ATTO-TEC

ATTO-TEC Fluorescent Labels and Dyes catalogue 2016/2018

Fluorescent Labels and Dyes

product catalogue

2016/2018

ATTO-TEC GmbH
Am Eichenhang 50
D-57076 Siegen
Germany

Phone: +49(0)-271-2 38 53-0
Fax: +49(0)-271-2 38 53-11
E-mail: sales@atto-tec.com
http: www.atto-tec.com

Contents

Success with Fluorescent Dyes

During the last two decades, there has been exceptional growth in the use of fluorescence in biological sciences. Fluorescence based methods, e.g. super-resolution microscopy – awarded with the Chemistry Nobel Prize in 2014 –, and time-resolved imaging, are considered to be primarily research tools in biochemistry and biophysics.

Since its start-up in 1999 ATTO-TEC has taken up the challenge to develop novel and innovative functional dyes for these sophisticated life science applications. The staff of ATTO-TEC has more than 40 years of experience in fluorescent dye chemistry. This experience and the passion for fluorescence is our motivation to become that little bit better every day, ensuring that we continuously meet or surpass the expectations of our customers.

The new edition of our catalogue describes some fundamental principles of fluorescence and should be a helpful guide to find the right label for your very special needs. Its content is also available and regularly updated on our website (www.atto-tec.com).

We are proud to present more than 40 labels and quenchers of the well known ATTO family. These dyes are proprietary compounds covered by patents and patent applications. They are all available with a large variety of different modifications, e.g. NHS-esters, maleimides, and other derivatives, for the coupling to biomolecules.

Researchers and companies all over the world appreciate our ATTO dyes for their exceptional purity and excellent performance. Continuous monitoring and improvement of all manufacturing processes guarantee the consistent high quality of every single product.

We would encourage you to address, together with us, the challenges and chances in the field of fluorescent applications.

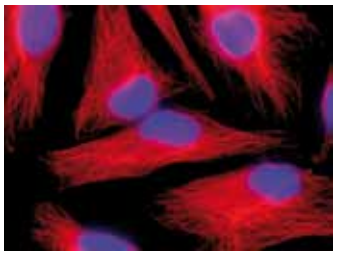
Your ATTO-TEC Team

ATTO-TEC

Contact Information

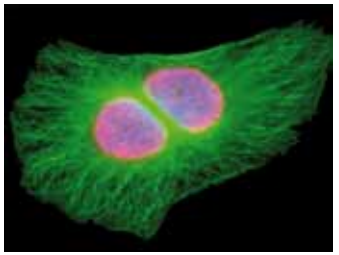
Addresses:

ATTO-TEC GmbH
Am Eichenhang 50
D-57076 Siegen
Germany



ATTO-TEC GmbH
P.O. Box 10 08 64
D-57008 Siegen
Germany

Phone: +49(0)-271-2 38 53-0
Fax: +49(0)-271-2 38 53-11
E-mail: info@atto-tec.com
http: www.atto-tec.com



To Place Orders:

Phone: +49(0)-271-2 38 53-0
Fax: +49(0)-271-2 38 53-11
E-mail: sales@atto-tec.com
http: www.atto-tec.com

Fluorescence

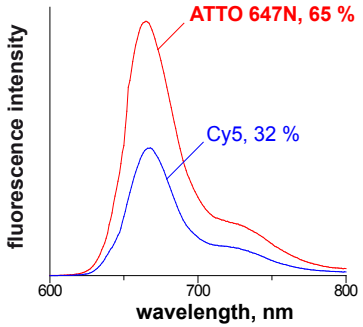
The emission of light by molecules, so-called fluorescence, has been known for more than one hundred and sixty years. Due to the development of versatile light sources (lasers etc.) and accurate detectors, fluorescence spectroscopy has become a powerful tool with outstanding sensitivity. By sophisticated techniques nowadays even single molecules can be studied via fluorescence. The great potential of such methods is emphasized by the 2014 Nobel Prize in Chemistry awarded for the invention of super-resolved fluorescence microscopy.

Most molecules of interest, e.g. in biochemistry, do not show fluorescence of their own. However, they may be chemically connected, i.e. labeled, with a fluorescent dye. Therefore the development of dyes that are suitable as labels is a subject of great importance in modern biology, medicine, and diagnostics.

How to Choose the Right Label

To obtain the best possible results several factors have to be considered. First is the source of excitation: To reduce interference due to autofluorescence of the sample an excitation wavelength above 550 nm or even 600 nm is preferable. Besides reduced background, the red spectral region is also advantageous when working with living cells, because damage is reduced.
Secondly the label should show strong absorption at the excitation wavelength, as well as high fluorescence quantum yield. The product of extinction coefficient and fluorescence quantum yield is often called the „brightness“ of a dye.

The fluorescence efficiency of dyes is highest in the blue and green region of the spectrum. Here the quantum yield reaches in some cases almost the theoretical limit of 100 %. Towards longer wavelengths the efficiency of the emission drops drastically, in particular so in aqueous solution. However, ATTO-TEC has been able to develop labels that show high quantum yield even around 650 nm. For instance: ATTO 647**N** (p. 53) fluoresces in aqueous solution twice as strong as the older cyanine dye Cy5™.



Finally the emission spectrum of the label should match the transmission of the applied filter set. The filter set, in turn, must be chosen such that it rejects the excitation light scattered by the sample, yet transmits the fluorescence as effectively as possible.

For example, when using a diode laser of wavelength 635 nm as excitation source and a filter set with high transmittance between 650 nm and 750 nm, ATTO 647**N** would be a very good choice. As can be seen from the list of ATTO-labels in this catalogue, ATTO647**N** has a high extinction coefficient at 635 nm, a wavelength close to the maximum of the absorption curve, as well as an excellent quantum yield of fluorescence (η_{fl} = 65 %).

The table below provides an overview of some frequently used excitation sources and recommended ATTO-labels.

Light source	Emission line	Suitable dyes
Mercury arc lamp	365 nm 405 nm 436 nm 546 nm 577 nm	ATTO 390 ATTO 425, ATTO 430LS ATTO 425, ATTO 430LS, ATTO 465 ATTO 550, ATTO 565 ATTO Rho12, ATTO Rho101, ATTO 590, ATTO Rho13, ATTO 594, ATTO 610, ATTO Rho14
Argon-Ion laser	488 nm 514 nm	ATTO 488, ATTO 490LS, ATTO 514, ATTO 520 ATTO 514, ATTO 490LS, ATTO 520, ATTO 532, ATTO 542
Nd:YAG laser, frequency doubled	532 nm	ATTO 532, ATTO Rho6G, ATTO 542, ATTO 550, ATTO 565, ATTO Rho11, ATTO Rho12
He-Ne laser	633 nm	ATTO 633, ATTO 647, ATTO 647**N**, ATTO 655
Krypton-Ion laser	647 nm 676 nm	ATTO 647, ATTO 647**N**, ATTO 655, ATTO Oxa12, ATTO 665, ATTO 680 ATTO 680, ATTO 700, ATTO 725, ATTO 740
Diode laser	635 nm	ATTO 633, ATTO 647, ATTO 647**N**, ATTO 655

Nowadays, compact and powerful laser diodes are covering the whole visible and near infrared part of the spectrum. They found their way into many applications/devices as very efficient excitation sources and more and more substitute classical light sources.

If there is no label available with an absorption maximum exactly matching the wavelength of the excitation source, a label with slightly longer wavelength should be chosen. The absorbance will be smaller, but the larger difference between excitation wavelength and fluorescence spectrum, which is always independent of excitation wavelength, has the advantage of better discrimination against scattered excitation light.

Properties of Fluorescent Labels

It is to be noted, however, that besides the already discussed optical considerations other factors may be important selecting a label, e.g. pH-dependence of the optical and chemical properties of the dye, solubility, photo- and chemical stability, size of chromophore or length of the linker, and several others concerning/matching the demands for the application in hand.
These properties can be highly relevant with respect to the suitability of dyes as labels. Most important, the dye must remain intact during irradiation. Many common labels, e.g. Fluorescein derivative FITC, show very low photostability. As a result sensitivity and quality of imaging are limited if high-intensity laser excitation is used and processes are to be observed over long periods of time. This is a serious draw-back with microscopy and other techniques based on the confocal principle, e.g. in single cell detection applications. In contrast to some widely used older dyes, the new patented ATTO-labels are designed to be much more stable under prolonged irradiation.

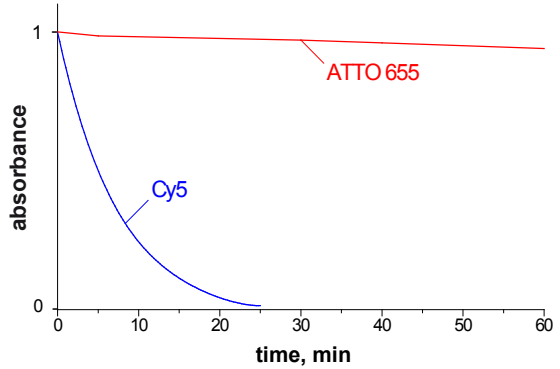


Photo-stability of ATTO 655 compared with common Cy5™ in water. Radiation of a 250 W tungsten-halogen lamp focussed into a 1 cm cell. Absorbance vs. time of illumination.

Many common fluorescent labels deteriorate even without any irradiation (i.e. in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the dyes ATTO 647**N** and ATTO 655 last up to 100 times longer than dyes like the cyanine dyes Cy5™ and Alexa647™. This is very important in microarray applications, where the dye molecules are located at the surface and thus are directly exposed to the atmosphere.

Förster Resonance Energy Transfer (FRET)

FRET is becoming increasingly important as a method to determine distances at the molecular level and to study dynamic processes like binding of antibody/antigen pairs. If two dye molecules are located close to each other, their transition dipoles can interact, and energy can be transferred from one dye molecule (donor) to the other (acceptor). The rate of energy transfer k_{ET} is according to Förster theory:

$$k_{ET} = \frac{9\,ln10}{128\,\pi^5} \cdot \frac{\kappa^2}{N_A\; n^4\; \tau_0\; r^6} \int_0^\infty F(\lambda) \cdot \varepsilon(\lambda) \cdot \lambda^4\, d\lambda$$

N_A	Avogadro constant
n	refractive index of solvent
τ_0	radiative decay time of donor
r	distance between donor and acceptor molecule
$F(\lambda)$	fluorescence spectrum of donor, normalized according to $\int_0^\infty F(\lambda)\, d\lambda = 1$
$\varepsilon(\lambda)$	molar decadic extinction coefficient of acceptor
κ^2	orientation factor: $\kappa^2 = (\cos\varphi_{DA} - 3\cos\varphi_D \cos\varphi_A)^2$
φ_{DA}	angle between transition dipoles of donor and acceptor
φ_D	angle between donor transition dipole and line connecting the dipoles
φ_A	angle between acceptor transition dipole and line connecting the dipoles

As can be seen from the formula, the rate of energy transfer decreases with the 6th power of the distance between the dye molecules. FRET is very efficient only when donor and acceptor are in close proximity. With typical dye molecules it becomes negligibly small at distances above 100 Å (10 nm). Furthermore its rate is proportional to the extinction coefficient of the acceptor dye in the wavelength range of the donor fluorescence (overlap integral): FRET is most efficient, if there is a good spectral overlap between fluorescence of donor and absorption of acceptor. A practical measure of FRET efficiency is the distance at which the rate k_{ET} of energy transfer equals the rate of donor fluorescence. This so-called Förster-radius R_0 is given by:

$$R_0^{\,6} = \frac{9\,ln10}{128\,\pi^5} \cdot \frac{\kappa^2\,\eta_{fl}}{N_A\;n^4} \int_0^\infty F(\lambda) \cdot\; \varepsilon(\lambda) \cdot\; \lambda^4\,d\lambda$$

η_{fl} fluorescence quantum yield of donor, $\eta_{fl} = \tau_{fl} / \tau_0$
τ_{fl} fluorescence decay time of donor

A table of Förster-radii for ATTO-dyes is presented on p. 14-17. These values have been calculated using the value n = 1.333 (refractive index of water) and with the assumption of statistical orientation of both donor and acceptor (orientation factor κ^2 = 2/3), a situation typically encountered in solutions of unbound dye molecules. However, in case of labeled biomolecules the chromophores of donor and acceptor may be held rigidly in fixed positions. As a consequence the orientation factor will assume a value different from 2/3. Since for κ^2 values between 0 and 4 are possible, the Förster-radius will vary accordingly. For accurate distance determinations via FRET it is vitally important to take the relative orientation of donor and acceptor into account.

Recommended donor-acceptor combinations of ATTO-labels according to the literature are:

ATTO 425 – ATTO 520
ATTO 488 – ATTO 550, ATTO 565, ATTO 647**N**, ATTO 655
ATTO 520 – ATTO 647**N**
ATTO 532 – ATTO 647**N**, ATTO 655
ATTO 550 – ATTO 590, ATTO 647**N**
ATTO 565 – ATTO 590, ATTO 647**N**
ATTO 590 – ATTO 620, ATTO 647**N**, ATTO 680
ATTO 620 – ATTO 680

Introduction

Selected Literature:

J. List, M. Weber, F. C. Simmel, *Hydrophobic Actuation of a DNA Origami Bilayer Structure*, Angew. Chem. Int. Ed. 53, 2014, 4236. ➔ **ATTO 532 – ATTO 647N**

H. Höfig, M. Gabba, S. Poblete, D. Kempe, J. Fitter, *Inter-Dye Distance Distributions Studied by a Combination of Single-Molecule FRET-Filtered Lifetime Measurements and a Weighted Accessible Volume (wAV) Algorithm*, Molecules 19, 2014, 19269.
➔ **ATTO 488 – ATTO 655**

T. E. Tomov, R. Tsukanov, M. Liber, R. Masoud, N. Plavner, E. Nir, *Rational Design of DNA Motors: Fuel Optimization through Single-Molecule Fluorescence*, J. Am. Chem. Soc. 135, 2013, 11935. ➔ **ATTO 550 – ATTO 647N**

E. Lerner, G. Hilzenrat, D. Amir, E. Tauber, Y. Garini, E. Haas, *Preparation of homogeneous samples of double-labelled protein suitable for single-molecule FRET measurements*, Anal. Bioanal. Chem. 405, 2013, 5983. ➔ **ATTO 488 – ATTO 647N**

S. Winterfeld, S. Ernst, M. Börsch, U. Gerken, A. Kuhn, *Real Time Observation of Single Membrane Protein Insertion Events by the Escherichia coli Insertase YidC*, PLoS ONE 8, 2013, e59023. ➔ **ATTO 520 – ATTO 647N**

S.L. Kuan, D.Y.W. Ng, Y. Wu, C. Förtsch, H. Barth, M. Doroshenko, K. Koynov, C. Meier, T. Weil, *pH Responsive Janus-like Supramolecular Fusion Proteins for Functional Protein Delivery*, J. Am. Chem. Soc. 135, 2013, 17254. ➔ **ATTO 425 – ATTO 520**

R. Bienert, B. Zimmermann, V. Rombach-Riegraf, P. Gräber, *Time-Dependent FRET with Single Enzymes: Domain Motions and Catalysis in H+-ATP Synthases*, ChemPhysChem, 12, 2011, 510. ➔ **ATTO 532 – ATTO 655**

K. Seyfert, T. Oosaka, H. Yaginuma, S. Ernst, H. Noji, R. Iino, M. Börsch, *Subunit rotation in a single FoF1-ATP synthase in living bacterium monitored by FRET*, Proc. SPIE 7905, 2011, 79050K-9. ➔ **ATTO 565 – ATTO 590 – ATTO 647N**

L. Marcon, C. Spriet, T. D. Meehan, B. J. Battersby, G. A. Lawrie, L. Héliot, M. Trau, *Synthesis and Application of FRET Nanoparticles in the Profiling of a Protease*, Small 5, 2009, 2053. ➔ **ATTO 488 – ATTO 550 – ATTO 590**

Introduction

Förster-radius R_0 of selected ATTO-dye pairs in Å (1 Å = 0.1 nm)

Donor	Acceptor																			
ATTO	390	425	430LS	465	488	490LS	495	514	520	532	Rho6G	540Q	542	550	565	Rho3B	Rho11	Rho12	575Q	Thio12
390	21	43	43	51	56	54	57	58	58	55	56	55	54	53	52	51	49	49	49	48
425		36	38	44	58	55	58	60	61	58	59	58	57	56	56	55	54	53	53	51
430LS			23	23	46	47	45	52	54	57	58	59	59	60	61	61	61	61	61	60
465				35	55	52	53	59	60	59	60	59	59	59	59	58	57	57	57	55
488					51	52	49	60	62	64	65	64	64	63	63	62	61	60	60	58
490LS						22	19	22	24	26	27	29	28	32	35	35	37	39	42	40
495							37	45	47	49	50	50	50	50	50	50	50	49	49	48
514								55	59	65	66	66	66	65	68	64	64	63	63	61
520									52	64	65	67	67	67	67	66	66	66	65	64
532										58	59	64	64	69	69	68	68	68	68	66
Rho6G											56	59	56	67	69	68	68	68	69	67
542													59	67	70	69	70	70	70	68
550														58	65	65	67	69	69	68
565															59	60	63	66	69	67
Rho3B																55	58	61	63	62
Rho11																	60	63	62	65
Rho12																		60	64	62

Acceptor																				Donor
580Q	Rho101	590	594	Rho13	610	612Q	620	Rho14	633	647	647N	655	Oxa12	665	680	700	725	740	ATTO
48	48	48	46	46	45	45	41	44	42	40	41	42	42	39	39	37	38	38	390
51	51	52	49	49	49	48	45	47	45	43	44	44	45	42	42	39	39	39	425
60	60	61	59	59	61	58	56	58	56	54	55	55	56	54	53	50	48	46	430LS
55	55	56	53	54	54	52	49	52	50	48	48	49	49	47	46	43	42	41	465
58	58	59	56	56	55	54	51	53	51	48	49	49	49	47	46	42	41	39	488
44	42	45	47	48	53	53	52	55	56	59	60	61	61	62	62	62	62	61	490LS
48	48	49	47	47	47	46	44	46	44	43	43	43	44	42	41	39	37	36	495
61	61	62	59	59	58	57	54	56	54	51	52	52	52	54	49	45	43	41	514
64	64	65	62	62	61	60	56	59	57	54	55	55	55	53	51	46	46	44	520
67	66	68	65	66	66	64	61	63	61	61	59	59	60	57	56	52	50	48	532
68	68	69	66	67	67	65	63	65	63	61	61	61	62	64	58	54	52	49	Rho6G
69	67	70	67	68	69	67	64	66	64	62	62	62	63	60	59	55	53	51	542
69	69	70	68	68	69	67	65	68	66	64	65	65	65	63	62	58	56	53	550
70	70	72	71	71	73	71	69	71	70	69	69	70	70	68	67	63	61	59	565
63	63	65	64	65	66	64	62	64	63	62	63	63	63	62	60	57	55	53	Rho3B
68	68	70	70	70	72	70	69	70	69	68	69	69	70	68	67	63	61	59	Rho11
66	66	69	70	70	73	71	70	71	70	69	70	70	71	70	68	65	62	60	Rho12

ATTO-TEC

Introduction

Förster-radius R_0 of selected ATTO-dye pairs in Å (1 Å = 0.1 nm)

Donor / Acceptor

ATTO	390	425	430LS	465	488	490LS	495	514	520	532	Rho6G	540Q	542	550	565	Rho3B	Rho11	Rho12	575Q	Thio12	580Q	Rho101	590	594	Rho13	610	612Q	620	Rho14	633	647	647N	655	Oxa12	665	680	700	725	740	ATTO
Thio12																			44	48	47	50	52	52	55	54	53	54	54	53	52	54	54	54	53	51	49	47	Thio12
Rho101																					61	66	69	69	74	72	71	73	72	71	72	72	72	71	70	67	67	62	Rho101
590																						60	65	65	72	71	70	73	73	72	73	73	74	73	72	70	68	65	590
594																							65	66	73	71	71	74	73	73	74	74	74	74	73	71	68	66	594
Rho13																								62	70	69	70	73	73	73	74	74	74	74	73	71	69	67	Rho13
610																									65	65	66	70	71	72	74	73	74	73	73	71	69	67	610
620																											59	63	65	68	70	70	70	70	69	68	67	65	620
Rho14																												67	70	74	76	76	76	77	75	74	73	71	Rho14
633																													63	69	71	73	73	74	73	72	72	70	633
647																														52	53	58	58	60	61	61	61	60	647
647N																															67	74	74	76	75	73	72	71	647N
655																																59	59	60	65	66	65	65	655
Oxa12																																	59	58	65	66	65	65	Oxa12
665																																		70	74	75	73	73	665
680																																			60	66	67	66	680
700																																				60	67	66	700
725																																					52	56	725
740																																						54	740

Fluorescence Quenchers

FRET from an excited dye molecule (donor) to another nearby dye molecule (acceptor) leads to the deactivation of the donor, its fluorescence is quenched. If the acceptor is fluorescent itself, it will emit light, exactly as if it had been directly excited. In contrast, if the acceptor is non-fluorescent, it will merely accept excitation energy from the donor, yet not produce any fluorescence by its own. Such acceptors are called „fluorescence quenchers“. ATTO-TEC provides quenchers covering the relevant visible spectrum. Their properties are outlined on p. 64-68.

Triplet Labels

On optical excitation of a dye molecule there is always a certain probability that the molecule is converted to the triplet state, a relatively long-lived, non-fluorescent excited state of the dye molecule. The occurrence of this state is frequently not desirable, as it promotes destruction (bleaching) of the dye. Nevertheless dyes with high triplet yield find application in photochemistry, photodynamic therapy etc. They are efficient sensitizers for the conversion of molecular oxygen (air) into its highly reactive form (singulet oxygen). In addition to the acridine dyes **ATTO 465** (p. 30) and **ATTO 495** (p. 32), both absorbing below 500 nm, we supply **ATTO Thio12** (p. 43), a triplet label derived from *Thiorhodamine* with an absorption maximum of 579 nm.

Redox Labels

A dye, well-known in biochemical and medical research, is Methylene Blue. It has very interesting redox properties: The dye, normally deep blue in color, is converted by mild reducing agents to its so-called leuko-form, which is colorless. Since this reaction is reversible, the blue color reappears on oxidation, e.g. by oxygen (air). ATTO-TEC offers **ATTO MB2** a *Methylene Blue* derivative featuring a carboxylic acid functionality for coupling (p. 63).

Introduction

Large Stokes-Shift Labels

The wavelength difference („Stokes-shift“) between absorption and fluorescence maximum of typical symmetrical dyes is about 20 – 30 nm. Coumarin dyes like **ATTO 390** (p. 28) and **ATTO 425** (p. 29) show a remarkably large Stokes-shift of about 90 and 50 nm. **ATTO 465** (p. 30) has a Stokes-shift of 55 nm in aqueous solution too. ATTO-TEC’s latest research led to a series of new dyes with an extraordinary large Stokes-shift of up to 163 nm: The dyes **ATTO 430LS** (p. 72) and **ATTO 490LS** (p. 73) are very hydrophilic and exhibit strong fluorescence in aqueous solution even after conjugation.

Fluorescence Labeled Membrane Probes

ATTO-TEC offers a variety of fluorescent labeled phospholipids. They are based on glycerol, carrying one or two fatty acids and a phosphate monoester residue for the investigation of biological membranes (p. 88)

ATTO-Dyes - Reactive Labels and Conjugates

The absorption spectra of the ATTO-labels – ranging from blue to the near-infrared – match the wavelengths of the most common excitation sources and are compatible with many established filters or typical instrument settings.

ATTO-labels are designed for application in the area of life science, e.g. for labeling of DNA, RNA or proteins. Characteristic features of most labels are strong absorption and high fluorescence efficiency („brightness“), excellent photo-stability, exceptionally high ozone resistance, and good water solubility.

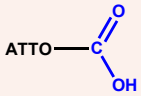
All ATTO-labels are available as NHS-esters for coupling to amino groups and as maleimides for coupling to thiol groups. In addition we offer most ATTO-dyes functionalized with iodoacetamide, amine or conjugated to phalloidin, streptavidin and biotin. The high affinity of streptavidin to biotin is the basis for the widespread use of streptavidin conjugates.

ATTO-dyes are also available functionalized as azide or alkyne for various applications in „Click Chemistry“. This concept describes pairs of functional groups reacting fast and selectively with each other under mild physiological conditions and being inert to naturally occurring functional groups such as amines („bioorthogonal“).

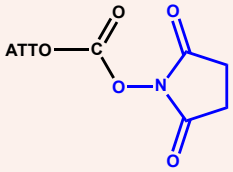
Introduction

ATTO Derivatives and Conjugates

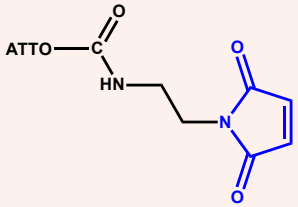
Carboxy:



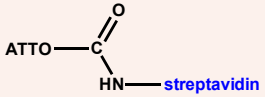
NHS-ester:



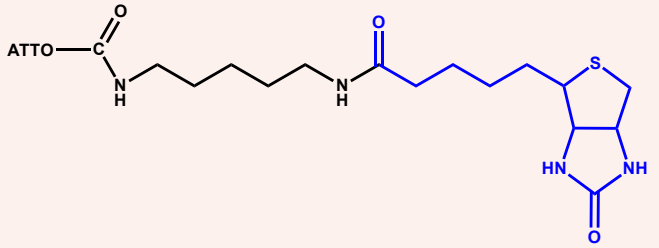
Maleimide:



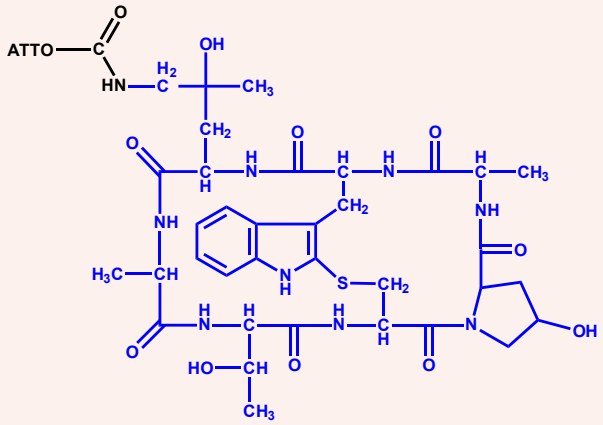
Streptavidin:



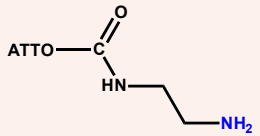
Biotin conjugate:

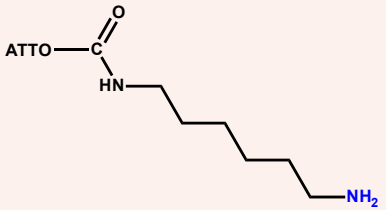


Phalloidin conjugate:

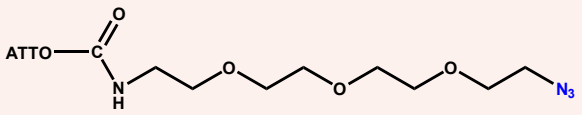


Amine:

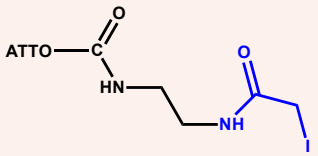




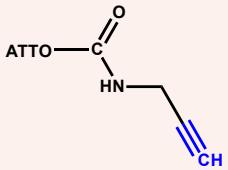
Azide:



Iodoacetamide:



Alkyne:

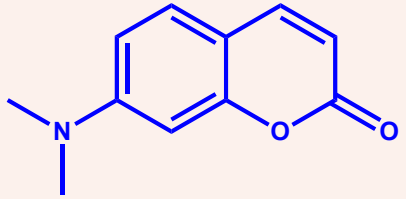


Molecular Structure of Fluorescent Labels

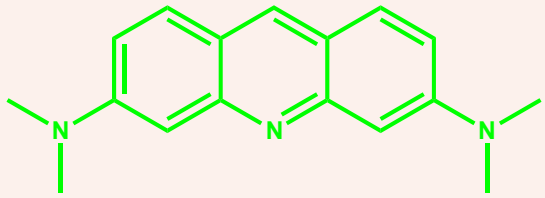
The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. In stark contrast to cyanines, ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature.

Most ATTO-labels are derivatives of:

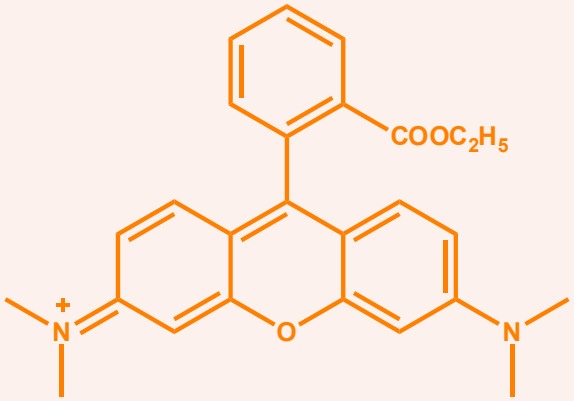
- **Coumarin**



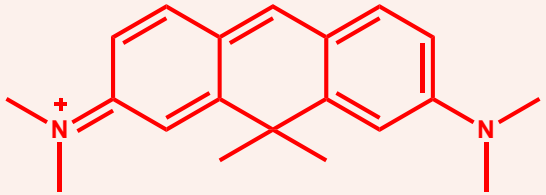
- **Acridine**



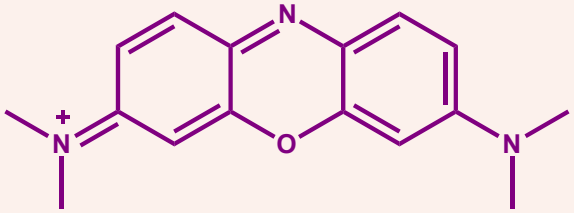
- **Rhodamine**



- **Carbopyronin**



- **Oxazine**



Introduction

About this Catalogue

All spectral data given have been measured at 22°C on aqueous solutions (PBS, pH 7.4) of the dyes with free carboxy group. When there was a tendency to aggregate, the solution was diluted sufficiently to exhibit the monomeric absorption spectrum undisturbed by dimers. Although water is the most important solvent in biochemistry, it should be borne in mind that optical data of dyes, in particular and most pronounced the fluorescence efficiency and decay time, depend on the solvent, as well as on other environmental factors. With most ATTO-dyes this influence is very weak indeed. Furthermore optical properties depend on the derivative (carboxy, NHS-ester, etc.). For instance, fluorescence quantum yield and decay time of the maleimide may be reduced compared to the dye with carboxy group (COOH). However, this is of no avail: As soon as the dye is coupled to a substrate (protein), the fluorescence is restituted.

The spectra presented in this catalogue will help to select the dye best suited for a particular experiment. For accurate data in digitized form the reader is referred to www.atto-tec.com (*Support - Downloads - Spectra)*. - The correction factors CF_{260} and CF_{280} aid in calculating the degree of labeling (DOL), see „Labeling Procedures“ (p. 76-85).

The molecular weight (*MW*) given has the common meaning, i.e. it refers to the dye including counterions (An^-). For mass spectrometry purposes the mass of the dye cation (M^+ or MH^+) is given. The value represents the mass of the signal of maximum intensity.

For further details on all products and for new developments please visit our website www.atto-tec.com.

ATTO-Labels
Optical Properties

ATTO Fluorescent Labels

Label	λ_{abs}, nm	ε_{max}, $M^{-1}\,cm^{-1}$	λ_{fl}, nm	η_{fl}, %	τ_{fl}, ns	Substitute for	Page
ATTO 390	390	24000	476	90	5.0		28
ATTO 425	439	45000	485	90	3.6		29
ATTO 430LS	436	32000	545	65	4.0		72
ATTO 465	453	75000	506	75	5.0		30
ATTO 488	500	90000	520	80	4.1	Alexa 488*, FITC, FAM**	31
ATTO 490LS	495	40000	658	30	2.6		73
ATTO 495	498	80000	526	20	1.0		32
ATTO 514	511	115000	532	85	3,9	Alexa 514	33
ATTO 520	517	110000	538	90	3.6	JOE**, TET**	34
ATTO 532	532	115000	552	90	3.8	Alexa 532*, HEX**	35
ATTO Rho6G	533	115000	557	90	4.1	HEX**	36
ATTO 542	542	120000	562	93	3.7		37
ATTO 550	554	120000	576	80	3.6	TAMRA**, Cy3***	38
ATTO 565	564	120000	590	90	4.0	Cy3.5***, ROX**	39
ATTO Rho3B	566	120000	589	50	1.5		40
ATTO Rho11	572	120000	595	80	4.0	ROX**	41
ATTO Rho12	576	120000	601	80	4.0		42
ATTO Thio12	582	110000	607	15	2.0		43
ATTO Rho101	587	120000	609	80	4.2		44
ATTO 590	593	120000	622	80	3.7	Alexa 594*, Texas Red*	45
ATTO 594	603	120000	626	85	3.9	Alexa 594*	46
ATTO Rho13	603	120000	627	80	3.9	Alexa 594*	47
ATTO 610	616	150000	633	70	3.2		48
ATTO 620	620	120000	642	50	2.9		49
ATTO Rho14	626	140000	646	80	3.7	Alexa 633*	50
ATTO 633	630	130000	651	64	3.3	Alexa 633*	51
ATTO 647	647	120000	667	20	2.4	Cy5***, Alexa 647*	52
ATTO 647**N**	646	150000	664	65	3.5	Cy5***, Alexa 647*	53
ATTO 655	663	125000	680	30	1.8	Cy5***, Alexa 647*	54
ATTO Oxa12	662	125000	681	30	1.8		55
ATTO 665	662	160000	680	60	2.9		56
ATTO 680	681	125000	698	30	1.7	Cy5.5***	57
ATTO 700	700	120000	716	25	1.6	Cy5.5***	58
ATTO 725	728	120000	751	10	0.5		59
ATTO 740	743	120000	763	10	0.6		60

ATTO Fluorescence Quenchers (p. 64)

Label	λ_{abs} nm	ε_{max} $M^{-1}\,cm^{-1}$	Quenching Range, nm
ATTO 540Q	543	105000	500 - 565
ATTO 575Q	582	120000	530 - 605
ATTO 580Q	587	110000	535 - 610
ATTO 612Q	615	115000	555 - 640

ATTO Large Stokes-Shift Dyes (p. 70)

Label	λ_{abs} nm	ε_{max} $M^{-1}\,cm^{-1}$	λ_{fl} nm	η_{fl} %
ATTO 390	390	24000	476	90
ATTO 425	439	45000	485	90
ATTO 465	453	75000	506	75
ATTO LS-Dye Series				
ATTO 430LS	436	32000	545	65
ATTO 490LS	495	40000	658	30

ATTO Triplet Labels

Label	λ_{abs} nm	ε_{max} $M^{-1}\,cm^{-1}$	λ_{fl} nm	η_T %	Page
ATTO 465	453	75000	506	10	30
ATTO 495	498	80000	526	10	32
ATTO Thio12	582	110000	607	20	43

ATTO Redox Label (p. 62)

Label	λ_{abs} nm	ε_{max} $M^{-1}\,cm^{-1}$
ATTO MB2	668	110000

λ_{abs}	longest-wavelength absorption maximum
ε_{max}	molar decadic extinction coefficient at the longest-wavelength absorption maximum
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	real fluorescence decay time
η_T	triplet quantum yield

All optical properties were measured in aqueous buffer solution (PBS, pH 7.4) at 22 °C and are valid for the carboxy derivative of each dye.

* Trademark of Invitrogen Corporation, ** Trademark of Applera Corporation, *** Trademark of GE Healthcare Group Companies

ATTO 390

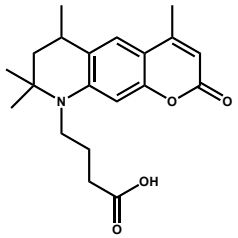
Optical properties of carboxy derivative

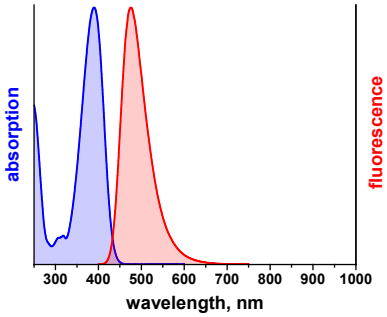
λ_{abs} = 390 nm
ε_{max} = 2.4 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 476 nm
η_{fl} = 90 %
τ_{fl} = 5.0 ns

CF_{260} = 0.46
CF_{280} = 0.09

Features:

- High fluorescence yield
- Large Stokes-shift
- Moderately hydrophilic
- Coumarin derivative, uncharged





Modification	MW, g/mol	MH^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	343	344	AD 390-21	AD 390-25
NHS-ester	440	441	AD 390-31	AD 390-35
maleimide	466	466	AD 390-41	AD 390-45
streptavidin			AD 390-61	AD 390-65
biotin	654	654	AD 390-71	AD 390-75
phalloidin	1113	1113	AD 390-81*	AD 390-82**
amine	500	386	AD 390-91	AD 390-95
azide	544	544	AD 390-101	AD 390-105
iodoacetamide	553	554	AD 390-111	AD 390-115
alkyne	495	381	AD 390-141	AD 390-145

* 10 nmol **20 nmol

ATTO 425

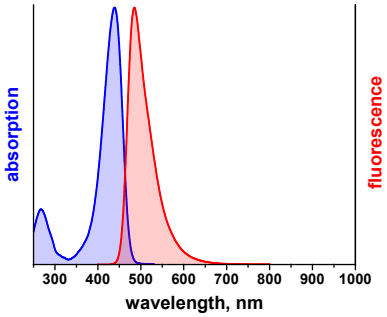
Optical properties of carboxy derivative

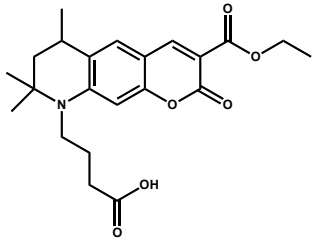
λ_{abs} = 439 nm
ε_{max} = 4.5 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 485 nm
η_{fl} = 90 %
τ_{fl} = 3.6 ns

CF_{260} = 0.19
CF_{280} = 0.17

Features:

- High fluorescence yield
- Large Stokes-shift
- Moderately hydrophilic
- Coumarin derivative, uncharged





Modification	MW, g/mol	MH^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	401	402	AD 425-21	AD 425-25
NHS-ester	499	499	AD 425-31	AD 425-35
maleimide	524	524	AD 425-41	AD 425-45
streptavidin			AD 425-61	AD 425-65
biotin	712	712	AD 425-71	AD 425-75
phalloidin	1171	1172	AD 425-81*	AD 425-82**
amine	558	444	AD 425-91	AD 425-95
azide	602	602	AD 425-101	AD 425-105

* 10 nmol **20 nmol

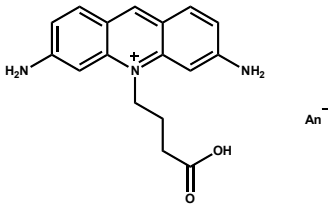
ATTO 465

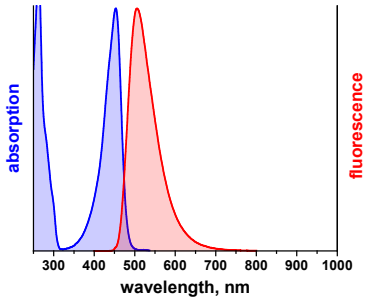
Optical properties of carboxy derivative

λ_{abs}	=	453 nm		
ε_{max}	=	7.5 x 10^4 M^{-1} cm^{-1}		
λ_{fl}	=	506 nm		
η_{fl}	=	75 %	CF_{260} =	1.09
η_T	=	10 %	CF_{280} =	0.48
τ_{fl}	=	5.0 ns		

Features:

- High fluorescence yield
- Large Stokes-shift in aqueous solution
- High triplet yield, intense phosphorescence in solid matrix
- Hydrophilic
- Cationic dye derived from well-known Acriflavine





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	396	296	AD 465-21	AD 465-25
NHS-ester	493	393	AD 465-31	AD 465-35
maleimide	518	418	AD 465-41	AD 465-45
streptavidin			AD 465-61	AD 465-65
biotin	706	606	AD 465-71	AD 465-75
phalloidin *new*	1179	1065	AD 465-81*	AD 465-82**

* 10 nmol **20 nmol

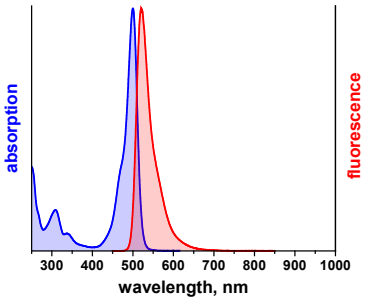
ATTO 488

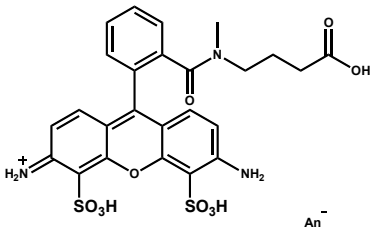
Optical properties of carboxy derivative

λ_{abs}	=	500 nm		
ε_{max}	=	9.0 x 10^4 M^{-1} cm^{-1}		
λ_{fl}	=	520 nm		
η_{fl}	=	80 %	CF_{260} =	0.22
τ_{fl}	=	4.1 ns	CF_{280} =	0.09

Features:

- High fluorescence yield
- High photo-stability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	804	590	AD 488-21	AD 488-25
NHS-ester	981	687	AD 488-31	AD 488-35
maleimide	1067	712	AD 488-41	AD 488-45
streptavidin			AD 488-61	AD 488-65
biotin	1191	900	AD 488-71	AD 488-75
phalloidin	1473	1359	AD 488-81*	AD 488-82**
amine	860	632	AD 488-91	AD 488-95
azide	904	790	AD 488-101	AD 488-105
iodoacetamide	914	800	AD 488-111	AD 488-115
alkyne	741	627	AD 488-141	AD 488-145

* 10 nmol **20 nmol

ATTO 495

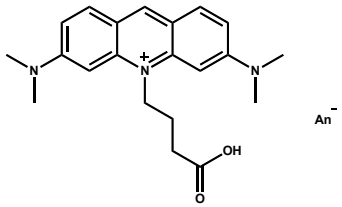
Optical properties of carboxy derivative

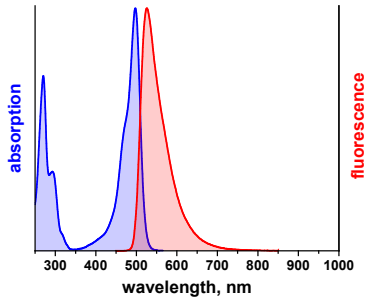
λ_{abs} = 498 nm
ε_{max} = 8.0 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 526 nm
η_{fl} = 20 %
η_T = 10 %
τ_{fl} = 1.0 ns

CF_{260} = 0.45
CF_{280} = 0.37

Features:

- High triplet yield
- Phosphorescent in solid matrix
- Penetrates the membrane of living cells
- Hydrophobic
- Cationic dye derived from well-known Acridine Orange





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	452	352	AD 495-21	AD 495-25
NHS-ester	549	449	AD 495-31	AD 495-35
maleimide	574	474	AD 495-41	AD 495-45
streptavidin *new*			AD 495-61	AD 495-65
biotin	762	662	AD 495-71	AD 495-75
phalloidin *new*	1235	1122	AD 495-81*	AD 495-82**

* 10 nmol **20 nmol

ATTO 514

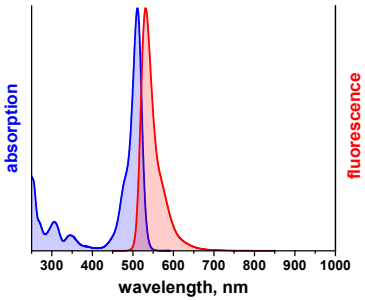
Optical properties of carboxy derivative

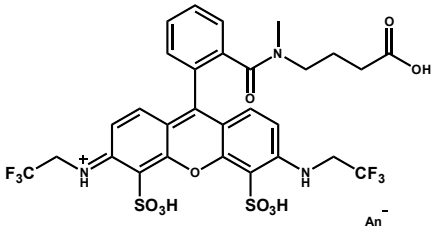
λ_{abs} = 511 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 532 nm
η_{fl} = 85 %
τ_{fl} = 3.9 ns

CF_{260} = 0.21
CF_{280} = 0.07

Features:

- High fluorescence yield
- High photo-stability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	868	754	AD 514-21	AD 514-25
NHS-ester	1111	851	AD 514-31	AD 514-35
maleimide	990	876	AD 514-41	AD 514-45
streptavidin			AD 514-61	AD 514-65
biotin	1178	1064	AD 514-71	AD 514-75
phalloidin	1638	1523	AD 514-81*	AD 514-82**
amine	1024	796	AD 514-91	AD 514-95
azide	1068	954	AD 514-101	AD 514-105
iodoacetamide	1078	964	AD 514-111	AD 514-115
alkyne	905	791	AD 514-141	AD 514-145

* 10 nmol **20 nmol

ATTO 520

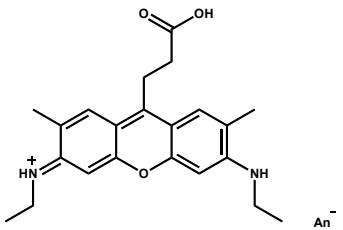
Optical properties of carboxy derivative

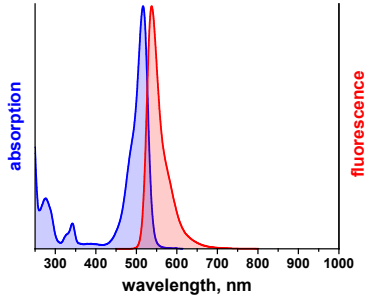
λ_{abs} = 517 nm
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 538 nm
η_{fl} = 90 %
τ_{fl} = 3.6 ns

CF_{260} = 0.16
CF_{280} = 0.20

Features:

- High fluorescence yield
- High thermal and photo-stability
- Hydrophobic
- At pH > 7 reversible formation of colorless pseudobase
- Cationic dye closely related to well-known Rhodamine 6G





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	467	367	AD 520-21	AD 520-25
NHS-ester	564	464	AD 520-31	AD 520-35
maleimide	589	489	AD 520-41	AD 520-45
biotin	777	677	AD 520-71	AD 520-75
phalloidin	1250	1136	AD 520-81*	AD 520-82**
amine	609	409	AD 520-91	AD 520-95
azide	681	567	AD 520-101	AD 520-105

* 10 nmol **20 nmol

ATTO 532

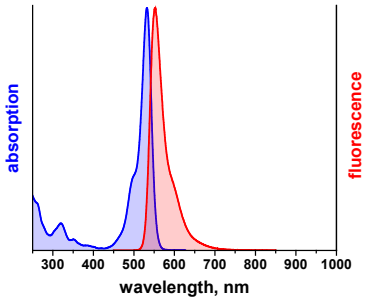
Optical properties of carboxy derivative

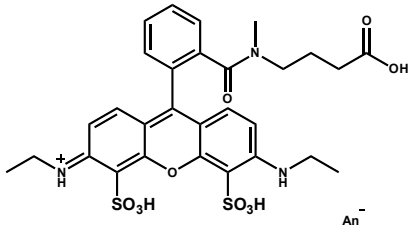
λ_{abs} = 532 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 552 nm
η_{fl} = 90 %
τ_{fl} = 3.8 ns

CF_{260} = 0.20
CF_{280} = 0.09

Features:

- High fluorescence yield
- High photo-stability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	765	646	AD 532-21	AD 532-25
NHS-ester	1081	743	AD 532-31	AD 532-35
maleimide	1063	768	AD 532-41	AD 532-45
streptavidin			AD 532-61	AD 532-65
biotin	1357	956	AD 532-71	AD 532-75
phalloidin	1530	1415	AD 532-81*	AD 532-82**
amine	916	688	AD 532-91	AD 532-95
azide	960	846	AD 532-101	AD 532-105
iodoacetamide	970	856	AD 532-111	AD 532-115
alkyne	797	683	AD 532-141	AD 532-145

* 10 nmol **20 nmol

ATTO Rho6G

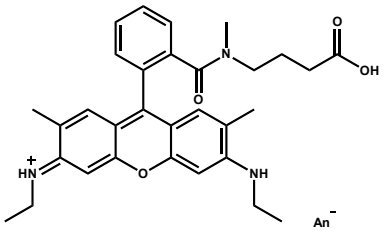
Optical properties of carboxy derivative

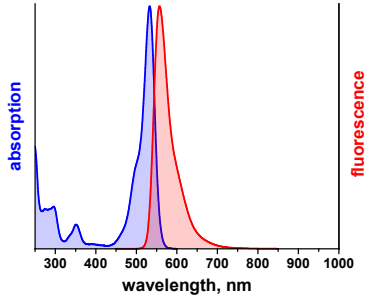
λ_{abs} = 533 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 557 nm
η_{fl} = 90 %
τ_{fl} = 4.1 ns

CF_{260} = 0.19
CF_{280} = 0.16

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye closely related to well-known Rhodamine 6G





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	614	514	AD Rho6G-21	AD Rho6G-25
NHS-ester	711	611	AD Rho6G-31	AD Rho6G-35
maleimide	750	636	AD Rho6G-41	AD Rho6G-45
biotin	938	824	AD Rho6G-71	AD Rho6G-75
phalloidin	1398	1283	AD Rho6G-81*	AD Rho6G-82**
amine	784	556	AD Rho6G-91	AD Rho6G-95
azide	828	714	AD Rho6G-101	AD Rho6G-105
alkyne	651	551	AD Rho6G-141	AD Rho6G-145

* 10 nmol **20 nmol

New ATTO 542

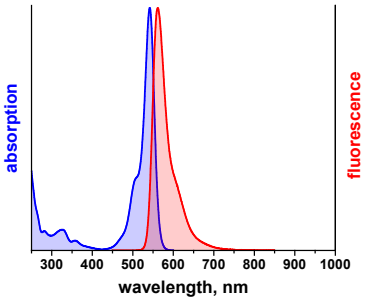
Optical properties of carboxy derivative

λ_{abs} = 542 nm
ε_{max} = 1.20 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 562 nm
η_{fl} = 93 %
τ_{fl} = 3.7 ns

CF_{260} = 0.18
CF_{280} = 0.08

Features:

- High fluorescence yield
- High photo-stability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)



Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	1028	914	AD 542-21	AD 542-25
NHS-ester	1125	1011	AD 542-31	AD 542-35
maleimide	1150	1036	AD 542-41	AD 542-45
streptavidin			AD 542-61	AD 542-65
phalloidin	1798	1683	AD 542-81*	AD 542-82**
azide	1228	1114	AD 542-101	AD 542-105

* 10 nmol **20 nmol

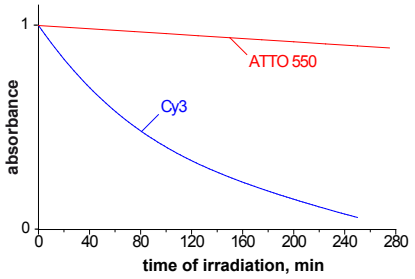
ATTO 550

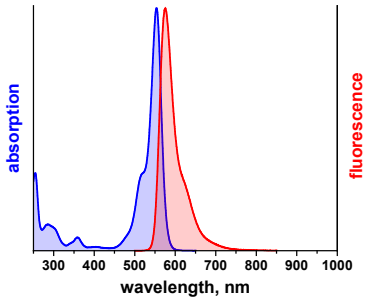
Optical properties of carboxy derivative

λ_{abs}	=	554 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	576 nm		
η_{fl}	=	80 %	CF_{260} =	0.23
τ_{fl}	=	3.6 ns	CF_{280} =	0.10

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye
- Supplied as mixture of three isomers with nearly identical properties





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	694	594	AD 550-21	AD 550-25
NHS-ester	791	691	AD 550-31	AD 550-35
maleimide	816	716	AD 550-41	AD 550-45
streptavidin			AD 550-61	AD 550-65
biotin	1005	904	AD 550-71	AD 550-75
phalloidin	1478	1363	AD 550-81*	AD 550-82**
amine	864	636	AD 550-91	AD 550-95
azide	908	794	AD 550-101	AD 550-105
iodoacetamide	918	804	AD 550-111	AD 550-115
alkyne	731	631	AD 550-141	AD 550-145

* 10 nmol **20 nmol

Fluorescent Labels
500 nm - 600 nm

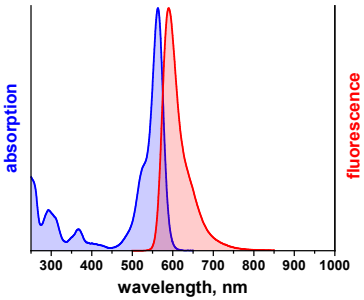
ATTO 565

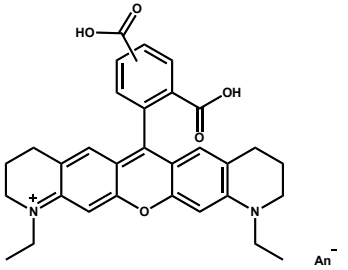
Optical properties of carboxy derivative

λ_{abs}	=	564 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	590 nm		
η_{fl}	=	90 %	CF_{260} =	0.27
τ_{fl}	=	4.0 ns	CF_{280} =	0.12

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Supplied as a mixture of two isomers with nearly identical properties
- Single isomer on request
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	611	511	AD 565-21	AD 565-25
NHS-ester	708	608	AD 565-31	AD 565-35
maleimide	733	633	AD 565-41	AD 565-45
streptavidin			AD 565-61	AD 565-65
biotin	922	821	AD 565-71	AD 565-75
phalloidin	1394	1280	AD 565-81*	AD 565-82**
amine	781	553	AD 565-91	AD 565-95
azide	825	711	AD 565-101	AD 565-105
iodoacetamide	835	721	AD 565-111	AD 565-115
alkyne	648	548	AD 565-141	AD 565-145

* 10 nmol **20 nmol

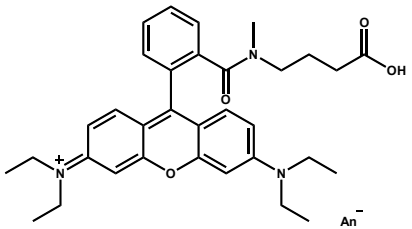
ATTO Rho3B

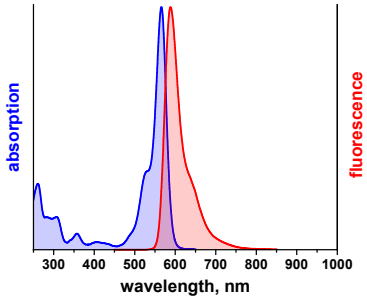
Optical properties of carboxy derivative

λ_{abs}	=	566 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	589 nm		
η_{fl}	=	50 % (in ethanol, 20°C)	CF_{260} =	0.27
τ_{fl}	=	1.5 ns	CF_{280} =	0.13

Features:

- Fluorescence yield strongly dependent on temperature
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye closely related to well-known Rhodamine B





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	642	542	AD Rho3B-21	AD Rho3B-25
NHS-ester	739	639	AD Rho3B-31	AD Rho3B-35
maleimide	764	664	AD Rho3B-41	AD Rho3B-45
biotin	966	852	AD Rho3B-71	AD Rho3B-75
phalloidin *new*	1426	1312	AD Rho3B-81*	AD Rho3B-82**

* 10 nmol **20 nmol

Fluorescent Labels
500 nm - 600 nm
ATTO-TEC

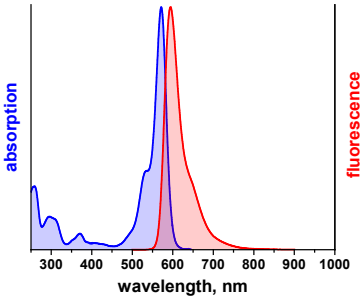
ATTO Rho11

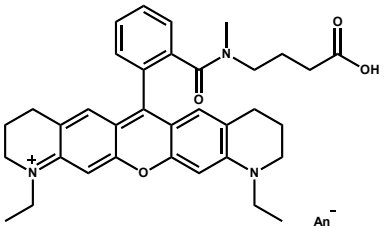
Optical properties of carboxy derivative

λ_{abs}	=	572 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	595 nm		
η_{fl}	=	80 %	CF_{260} =	0.26
τ_{fl}	=	4.0 ns	CF_{280} =	0.10

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	666	566	AD Rho11-21	AD Rho11-25
NHS-ester	763	663	AD Rho11-31	AD Rho11-35
maleimide	788	688	AD Rho11-41	AD Rho11-45
biotin	990	876	AD Rho11-71	AD Rho11-75
phalloidin *new*	1450	1336	AD Rho11-81*	AD Rho11-82**

* 10 nmol **20 nmol

ATTO Rho12

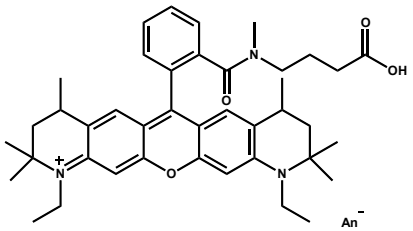
Optical properties of carboxy derivative

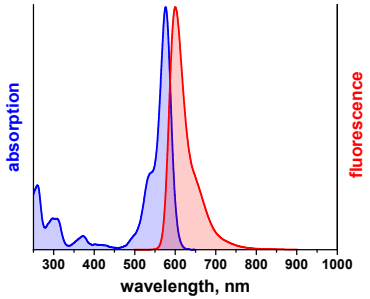
λ_{abs} = 577 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 600 nm
η_{fl} = 80 %
τ_{fl} = 4.0 ns

CF_{260} = 0.26
CF_{280} = 0.09

Features:

- High fluorescence yield
- High thermal and photo-stability
- Cationic dye
- Moderately hydrophilic
- Supplied as mixture of three isomers with nearly identical properties





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	750	650	AD Rho12-21	AD Rho12-25
NHS-ester	847	747	AD Rho12-31	AD Rho12-35
maleimide	872	772	AD Rho12-41	AD Rho12-45
biotin	1074	960	AD Rho12-71	AD Rho12-75
phalloidin *new*	1530	1416	AD Rho12-81*	AD Rho12-82**

* 10 nmol **20 nmol

ATTO Thio12

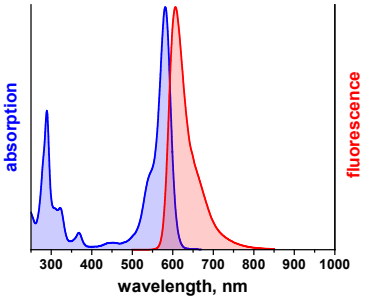
Optical properties of carboxy derivative

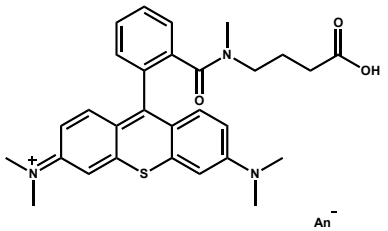
λ_{abs} = 582 nm
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 607 nm
η_{fl} = 15 %
η_T = 20 %
τ_{fl} = 2.0 ns

CF_{260} = 0.11
CF_{280} = 0.37

Features:

- High triplet yield
- High thermal stability
- Moderate fluorescence yield
- Cationic dye
- Moderately hydrophilic





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	602	502	AD Thio12-21	AD Thio12-25
NHS-ester	699	599	AD Thio12-31	AD Thio12-35
maleimide	724	624	AD Thio12-41	AD Thio12-45
biotin	926	812	AD Thio12-71	AD Thio12-75
phalloidin *new*	1386	1271	AD Thio12-81*	AD Thio12-82**

* 10 nmol **20 nmol

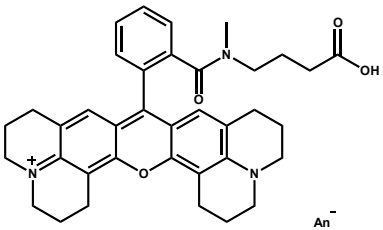
ATTO Rho101

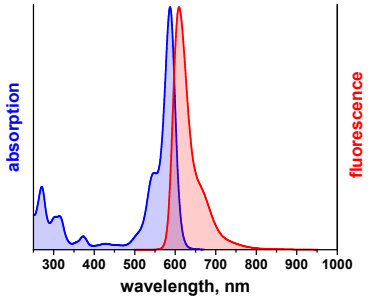
Optical properties of carboxy derivative

λ_{abs}	=	587 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	609 nm		
η_{fl}	=	80 %	CF_{260} =	0.18
τ_{fl}	=	4.2 ns	CF_{280} =	0.17

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Rhodamine dye related to well-known Rhodamine 101





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	703	590	AD Rho101-21	AD Rho101-25
NHS-ester	787	687	AD Rho101-31	AD Rho101-35
maleimide	812	712	AD Rho101-41	AD Rho101-45
biotin	1014	900	AD Rho101-71	AD Rho101-75
phalloidin *new*	1474	1360	AD Rho101-81*	AD Rho101-82**

* 10 nmol **20 nmol

Fluorescent Labels
500 nm - 600 nm

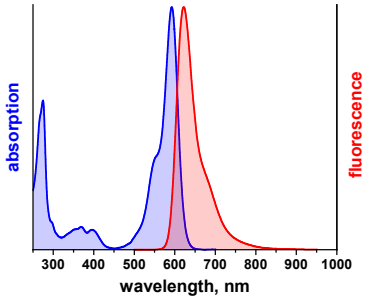
ATTO 590

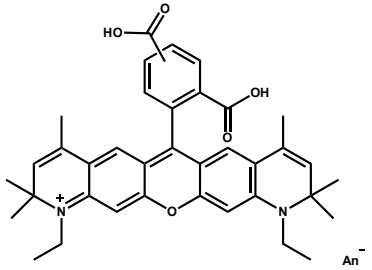
Optical properties of carboxy derivative

λ_{abs}	=	593 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	622 nm		
η_{fl}	=	80 %	CF_{260} =	0.39
τ_{fl}	=	3.7 ns	CF_{280} =	0.43

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	691	591	AD 590-21	AD 590-25
NHS-ester	788	688	AD 590-31	AD 590-35
maleimide	813	713	AD 590-41	AD 590-45
streptavidin			AD 590-61	AD 590-65
biotin	1002	901	AD 590-71	AD 590-75
phalloidin	1475	1360	AD 590-81*	AD 590-82**
amine#	917	689	AD 590-91	AD 590-95
azide	905	791	AD 590-101	AD 590-105
iodoacetamide#	971	857	AD 590-111	AD 590-115
alkyne	742	628	AD 590-141	AD 590-145

* 10 nmol **20 nmol # linker: hexamethylenediamine

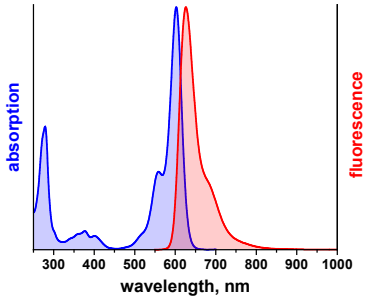
ATTO 594

Optical properties of carboxy derivative

λ_{abs}	=	603 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	626 nm		
η_{fl}	=	85 %	CF_{260} =	0.22
τ_{fl}	=	3.9 ns	CF_{280} =	0.50

Features:

- High fluorescence yield
- High photo-stability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)
- Net charge of -1



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	1137	806	AD 594-21	AD 594-25
NHS-ester	1389	903	AD 594-31	AD 594-35
maleimide	1358	928	AD 594-41	AD 594-45
streptavidin			AD 594-61	AD 594-65
biotin	1456	1116	AD 594-71	AD 594-75
phalloidin	1688	1575	AD 594-81*	AD 594-82**
amine	1076	848	AD 594-91	AD 594-95
azide	1119	1006	AD 594-101	AD 594-105
iodoacetamide	1129	1016	AD 594-111	AD 594-115
alkyne	956	843	AD 594-141	AD 594-145

* 10 nmol **20 nmol

Fluorescent Labels
500 nm - 600 nm

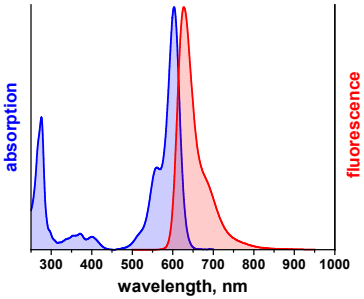
ATTO Rho13

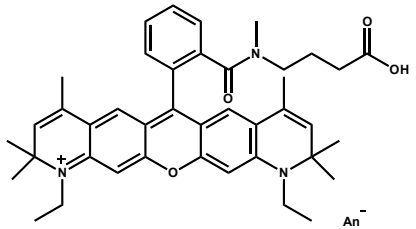
Optical properties of carboxy derivative

λ_{abs}	=	603 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	627 nm		
η_{fl}	=	80 %	CF_{260} =	0.28
τ_{fl}	=	3.9 ns	CF_{280} =	0.43

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	746	646	AD Rho13-21	AD Rho13-25
NHS-ester	843	743	AD Rho13-31	AD Rho13-35
maleimide	868	768	AD Rho13-41	AD Rho13-45
biotin	1070	956	AD Rho13-71	AD Rho13-75
phalloidin *new*	1530	1416	AD Rho13-81*	AD Rho13-82**

* 10 nmol **20 nmol

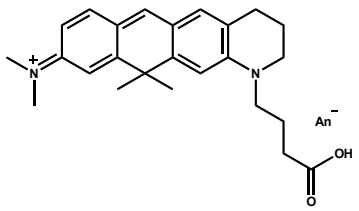
ATTO 610

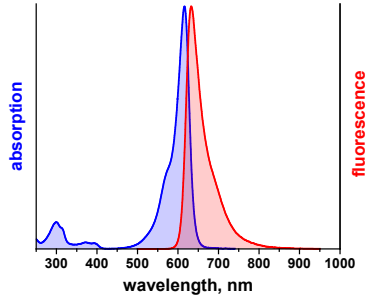
Optical properties of carboxy derivative

λ_{abs} = 616 nm
ε_{max} = 1.5 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 633 nm
η_{fl} = 70 %
τ_{fl} = 3.2 ns
CF_{260} = 0.03
CF_{280} = 0.06

Features:

- High fluorescence yield
- High photo-stability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Carbopyronin dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	491	391	AD 610-21	AD 610-25
NHS-ester	588	488	AD 610-31	AD 610-35
maleimide	613	513	AD 610-41	AD 610-45
streptavidin			AD 610-61	AD 610-65
biotin	801	701	AD 610-71	AD 610-75
phalloidin *new*	1274	1161	AD 610-81*	AD 610-82**

* 10 nmol **20 nmol

Fluorescent Labels
600 nm - 700 nm

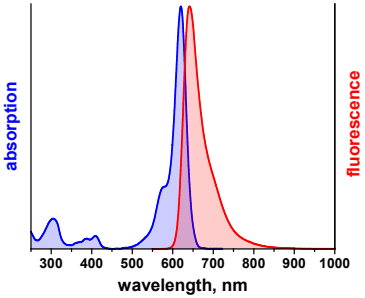
ATTO 620

Optical properties of carboxy derivative

λ_{abs} = 620 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 642 nm
η_{fl} = 50 %
τ_{fl} = 2.9 ns
CF_{260} = 0.04
CF_{280} = 0.06

Features:

- Fluorescence yield strongly dependent on temperature
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	612	512	AD 620-21	AD 620-25
NHS-ester	709	609	AD 620-31	AD 620-35
maleimide	734	634	AD 620-41	AD 620-45
streptavidin			AD 620-61	AD 620-65
biotin	923	822	AD 620-71	AD 620-75
phalloidin *new*	1396	1282	AD 620-81*	AD 620-82**

* 10 nmol **20 nmol

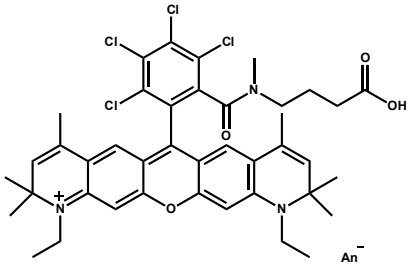
ATTO Rho14

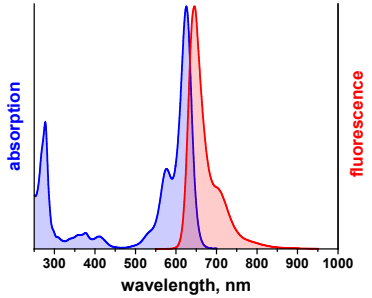
Optical properties of carboxy derivative

λ_{abs}	=	626 nm		
ε_{max}	=	1.4 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	646 nm		
η_{fl}	=	80 %	CF_{260} =	0.26
τ_{fl}	=	3.7 ns	CF_{280} =	0.47

Features:

- Extraordinary high fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	897	784	AD Rho14-21	AD Rho14-25
NHS-ester	981	881	AD Rho14-31	AD Rho14-35
maleimide	1020	906	AD Rho14-41	AD Rho14-45
biotin	1208	1094	AD Rho14-71	AD Rho14-75
phalloidin *new*	1668	1552	AD Rho14-81*	AD Rho14-82**

* 10 nmol **20 nmol

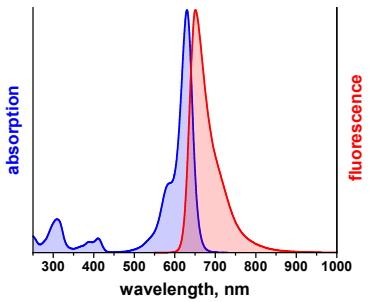
ATTO 633

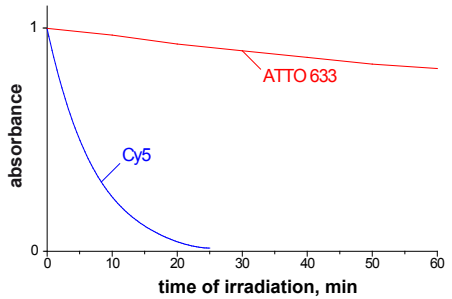
Optical properties of carboxy derivative

λ_{abs}	=	630 nm		
ε_{max}	=	1.3 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	651 nm		
η_{fl}	=	64 %	CF_{260} =	0.04
τ_{fl}	=	3.3 ns	CF_{280} =	0.05

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	652	552	AD 633-21	AD 633-25
NHS-ester	749	649	AD 633-31	AD 633-35
maleimide	774	674	AD 633-41	AD 633-45
streptavidin			AD 633-61	AD 633-65
biotin	963	862	AD 633-71	AD 633-75
phalloidin	1436	1321	AD 633-81*	AD 633-82**
amine	822	594	AD 633-91	AD 633-95
azide	866	752	AD 633-101	AD 633-105
iodoacetamide	876	762	AD 633-111	AD 633-115
alkyne	703	589	AD 633-141	AD 633-145

* 10 nmol **20 nmol

ATTO 647

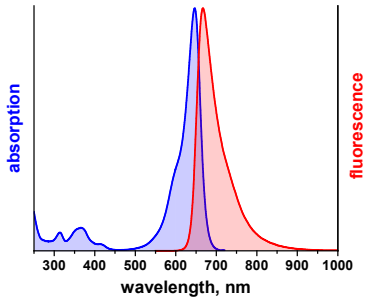
Optical properties of carboxy derivative

λ_{abs} = 647 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 667 nm
η_{fl} = 20 %
τ_{fl} = 2.4 ns

CF_{260} = 0.08
CF_{280} = 0.04

Features:

- High fluorescence yield
- High photo-stability
- Hydrophilic
- Stable at pH 2 - 8
- Zwitterionic dye



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	593	593	AD 647-21	AD 647-25
NHS-ester	811	690	AD 647-31	AD 647-35
maleimide	829	715	AD 647-41	AD 647-45
streptavidin			AD 647-61	AD 647-65
biotin	1219	903	AD 647-71	AD 647-75
phalloidin	1477	1363	AD 647-81*	AD 647-82**

* 10 nmol **20 nmol

ATTO 647N

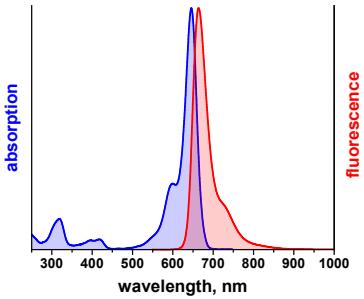
Optical properties of carboxy derivative

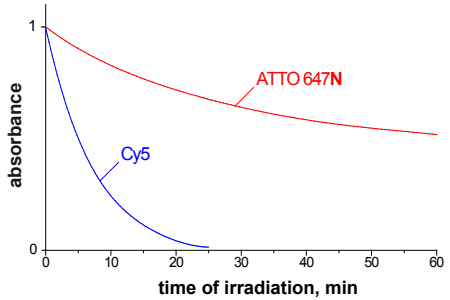
λ_{abs} = 646 nm
ε_{max} = 1.5 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 664 nm
η_{fl} = 65 %
τ_{fl} = 3.5 ns

CF_{260} = 0.04
CF_{280} = 0.03

Features:

- Extraordinary high fluorescence yield
- High thermal and photo-stability
- Excellent ozone resistance
- Moderately hydrophilic
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)
- Cationic dye, mixture of two isomers





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	746	646	AD 647**N**-21	AD 647**N**-25
NHS-ester	843	743	AD 647**N**-31	AD 647**N**-35
maleimide	868	768	AD 647**N**-41	AD 647**N**-45
streptavidin			AD 647**N**-61	AD 647**N**-65
biotin	1057	956	AD 647**N**-71	AD 647**N**-75
phalloidin	1530	1415	AD 647**N**-81*	AD 647**N**-82**
amine	916	688	AD 647**N**-91	AD 647**N**-95
azide	960	846	AD 647**N**-101	AD 647**N**-105
iodoacetamide	970	856	AD 647**N**-111	AD 647**N**-115
alkyne	783	683	AD 647**N**-141	AD 647**N**-145

* 10 nmol **20 nmol

ATTO 655

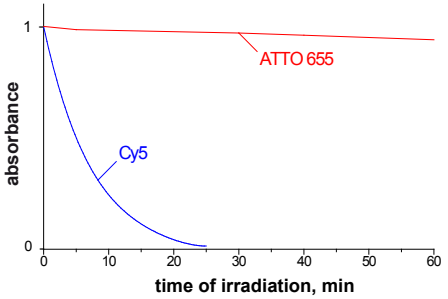
Optical properties of carboxy derivative

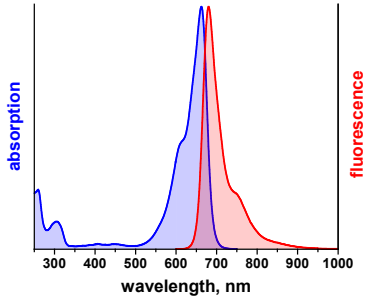
λ_{abs} = 663 nm
ε_{max} = 1.25 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 680 nm
η_{fl} = 30 %
τ_{fl} = 1.8 ns

CF_{260} = 0.24
CF_{280} = 0.08

Features:

- High fluorescence yield
- Excellent thermal and photo-stability
- Excellent ozone resistance
- Hydrophilic
- Zwitterionic dye
- Highly suitable for single-molecule applications and high-resolution microscopy (STED, dSTORM, etc.)
- Fluorescence quenching by guanine, tryptophan, etc.





Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	634	528	AD 655-21	AD 655-25
NHS-ester	887	625	AD 655-31	AD 655-35
maleimide	812	650	AD 655-41	AD 655-45
streptavidin			AD 655-61	AD 655-65
biotin	1204	838	AD 655-71	AD 655-75
phalloidin	1412	1297	AD 655-81*	AD 655-82**
amine	798	570	AD 655-91	AD 655-95
azide	842	728	AD 655-101	AD 655-105
iodoacetamide	852	738	AD 655-111	AD 655-115
alkyne	679	565	AD 655-141	AD 655-145

* 10 nmol **20 nmol

Fluorescent Labels
600 nm - 700 nm

ATTO Oxa12

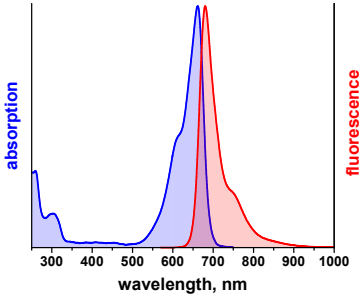
Optical properties of carboxy derivative

λ_{abs} = 662 nm
ε_{max} = 1.25 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 681 nm
η_{fl} = 30 %
τ_{fl} = 1.8 ns

CF_{260} = 0.32
CF_{280} = 0.12

Features:

- High fluorescence yield
- High thermal and photo-stability
- Lipophilic variety of ATTO 655
- Good solubility in organic solvents of medium polarity
- Cationic dye



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	739	639	AD Oxa12-21	AD Oxa12-25
NHS-ester	835	736	AD Oxa12-31	AD Oxa12-35
maleimide	875	761	AD Oxa12-41	AD Oxa12-45
streptavidin *new*			AD Oxa12-61	AD Oxa12-65
biotin	1063	949	AD Oxa12-71	AD Oxa12-75
phalloidin *new*	1523	1409	AD Oxa12-81*	AD Oxa12-82**

* 10 nmol **20 nmol

ATTO 665

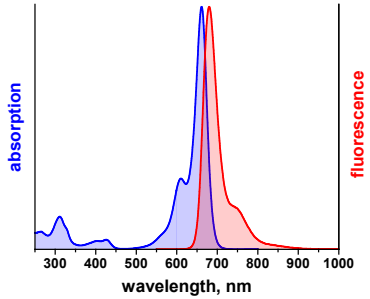
Optical properties of carboxy derivative

λ_{abs} = 662 nm
ε_{max} = 1.60 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 680 nm
η_{fl} = 60 %
τ_{fl} = 2.9 ns

CF_{260} = 0.07
CF_{280} = 0.06

Features:

- Extraordinary high fluorescence yield
- Excellent thermal and photo-stability
- Excellent ozone resistance
- Hydrophobic
- Cationic dye



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	723	623	AD 665-21	AD 665-25
NHS-ester	820	720	AD 665-31	AD 665-35
maleimide	845	745	AD 665-41	AD 665-45
streptavidin			AD 665-61	AD 665-65
biotin	1046	933	AD 665-71	AD 665-75
phalloidin	1507	1392	AD 665-81*	AD 665-82**
azide	937	823	AD 665-101	AD 665-105

* 10 nmol **20 nmol

ATTO 680

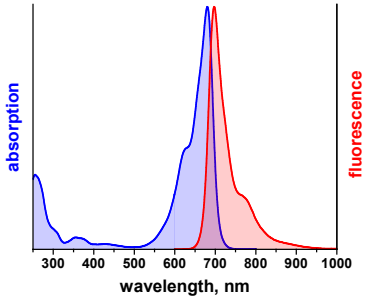
Optical properties of carboxy derivative

λ_{abs} = 681 nm
ε_{max} = 1.25 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 698 nm
η_{fl} = 30 %
τ_{fl} = 1.7 ns

CF_{260} = 0.30
CF_{280} = 0.17

Features:

- High fluorescence yield
- Excellent thermal and photo-stability
- Fluorescence quenching by guanine, tryptophan, etc.
- Hydrophilic
- Zwitterionic dye



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	631	526	AD 680-21	AD 680-25
NHS-ester	828	623	AD 680-31	AD 680-35
maleimide	1024	648	AD 680-41	AD 680-45
streptavidin			AD 680-61	AD 680-65
biotin	1123	836	AD 680-71	AD 680-75
phalloidin	1410	1295	AD 680-81*	AD 680-82**
amine	796	568	AD 680-91	AD 680-95
azide	839	726	AD 680-101	AD 680-105
iodoacetamide	850	736	AD 680-111	AD 680-115
alkyne	677	563	AD 680-141	AD 680-145

* 10 nmol **20 nmol

ATTO 700

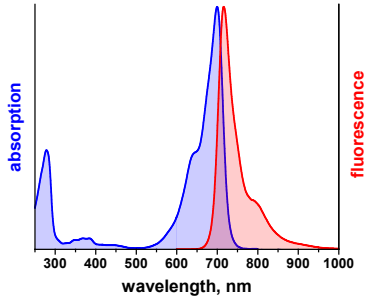
Optical properties of carboxy derivative

λ_{abs} = 700 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 716 nm
η_{fl} = 25 %
τ_{fl} = 1.6 ns

CF_{260} = 0.26
CF_{280} = 0.41

Features:

- High fluorescence yield
- Excellent thermal and photo-stability
- Fluorescence quenching by guanine, tryptophan, etc.
- Hydrophilic
- Zwitterionic dye



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	575	566	AD 700-21	AD 700-25
NHS-ester	837	663	AD 700-31	AD 700-35
maleimide	971	688	AD 700-41	AD 700-45
streptavidin			AD 700-61	AD 700-65
biotin	973	876	AD 700-71	AD 700-75
palloidin	1450	1335	AD 700-81*	AD 700-82**
amine	836	608	AD 700-91	AD 700-95
azide	880	766	AD 700-101	AD 700-105
alkyne	717	603	AD 700-141	AD 700-145

* 10 nmol **20 nmol

Fluorescent Labels
700 nm - 750 nm

ATTO 725

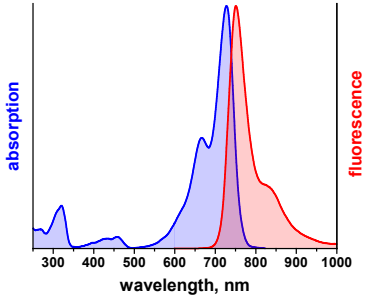
Optical properties of carboxy derivative

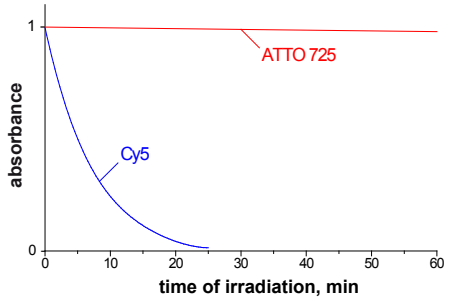
λ_{abs} = 728 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 751 nm
η_{fl} = 10 %
τ_{fl} = 0.5 ns

CF_{260} = 0.08
CF_{280} = 0.06

Features:

- Excellent photo-stability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Cationic dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	516	416	AD 725-21	AD 725-25
NHS-ester	613	513	AD 725-31	AD 725-35
maleimide	638	538	AD 725-41	AD 725-45
streptavidin *new*			AD 725-61	AD 725-65
biotin	826	726	AD 725-71	AD 725-75
phalloidin	1299	1185	AD 725-81*	AD 725-82**
azide	729	616	AD 725-101	AD 725-105

* 10 nmol **20 nmol

ATTO 740

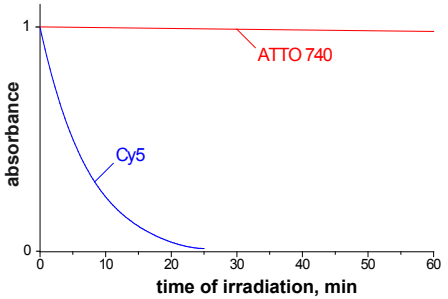
Optical properties of carboxy derivative

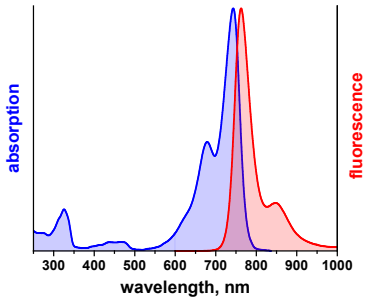
λ_{abs} = 743 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 763 nm
η_{fl} = 10 %
τ_{fl} = 0.6 ns

CF_{260} = 0.07
CF_{280} = 0.07

Features:

- Excellent photo-stability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Cationic dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	568	468	AD 740-21	AD 740-25
NHS-ester	665	565	AD 740-31	AD 740-35
maleimide	690	590	AD 740-41	AD 740-45
streptavidin *new*			AD 740-61	AD 740-65
biotin	879	778	AD 740-71	AD 740-75
phalloidin	1352	1237	AD 740-81*	AD 740-82**
azide	782	668	AD 740-101	AD 740-105

* 10 nmol **20 nmol

New Cyan 500

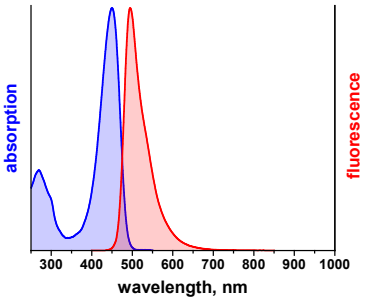
Optical properties

λ_{abs} = 450 nm
ε_{max} = 4.5 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 495 nm
η_{fl} = 85 %

CF_{260} = 0.31
CF_{280} = 0.30

Features:

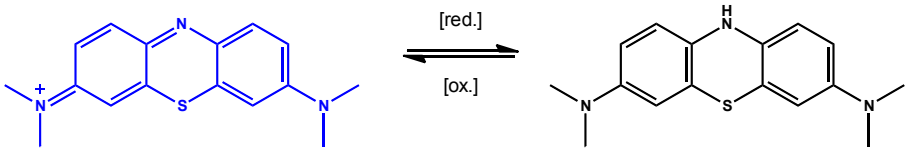
- High fluorescence yield
- Large Stokes-shift
- Moderately hydrophilic
- Coumarin derivative, uncharged
- Designed and recommended by Roche for application in LightCycler® instruments



Modification	MW, g/mol	MH^+, g/mol	Order Code 1 mg	Order Code 5 mg
NHS-ester	580	581	Cyan500-31	Cyan500-35

Redox Label

A dye, well-known in biochemical and medical research, is *Methylene Blue*. It has very interesting redox properties: The dye, normally deep blue in color, is converted by mild reducing agents to its so-called *leuko*-form, which is colorless. Since this reaction is reversible, the blue color reappears on oxidation, e.g. by oxygen (air). These interconversions can be catalyzed enzymatically.



Methylene Blue
blue

***leuko*-form**
colorless

Methylene Blue as such cannot be coupled to biomolecules, because it lacks the necessary reactive groups. However, **ATTO-TEC** now offers **ATTO MB2**, a derivative of Methylene Blue. The dye is available as NHS-ester or maleimide for coupling to amino or thiol groups, respectively. **ATTO MB2** is also supplied as biotin conjugate for direct coupling to avidin or streptavidin.

ATTO MB2

Optical properties of carboxy derivative

λ_{abs} = 668 nm

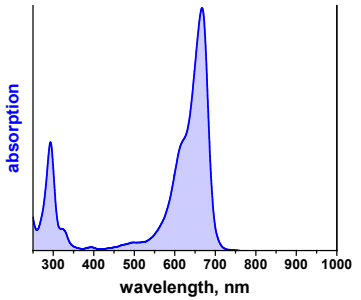
ε_{max} = 1.00 x 10^5 M^{-1} cm^{-1}

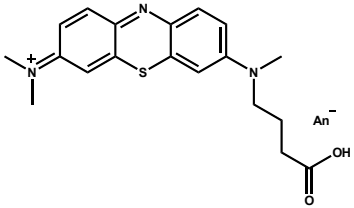
CF_{260} = 0.08

CF_{280} = 0.24

Features:

- High thermal and photo-stability
- Redox Label
- Moderately hydrophilic
- Cationic dye





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	392	356	AD MB2-21	AD MB2-25
NHS-ester	553	453	AD MB2-31	AD MB2-35
maleimide	591	478	AD MB2-41	AD MB2-45
streptavidin			AD MB2-61	AD MB2-65
biotin	779	666	AD MB2-71	AD MB2-75
phalloidin *new*	1239	1125	AD MB2-81*	AD MB2-82**

* 10 nmol **20 nmol

Förster resonance energy transfer (FRET) from an excited dye molecule (donor) to another nearby dye molecule (acceptor) leads to deactivation of the donor, i.e. it no longer fluoresces: Its fluorescence is *quenched*. The process of FRET depends, among other factors, on the absorption spectrum of the acceptor, as was discussed in some detail on p. 11-13. If the acceptor is *fluorescent* itself, it will emit light just the same, as if it had been excited directly (without utilisation of the donor). However, if the acceptor is *non-fluorescent*, it will merely accept excitation energy from the donor, yet not produce any fluorescence by its own. Such acceptors are called "**fluorescence quenchers**".

Fluorescence quenchers reduce the fluorescence intensity of the donor dye according to the formulas given on p. 11-12. The Förster-radius R_0 is determined by the overlap between fluorescence spectrum of the donor and absorption spectrum of the acceptor (quencher). For efficient quenching the absorption region of the quencher must overlap well with the fluorescence spectrum of the donor.

ATTO-TEC provides quenchers covering most of the relevant visible spectrum. Their properties are outlined on p. 65-68. The Förster-radii R_0 for combinations with fluorescent ATTO-labels as donors are presented in the table on p. 14-17.

Note:

1. The fluorescence of dyes may be quenched also by mechanisms entirely different than FRET. For example, the fluorescence of **ATTO 655**, **ATTO 680**, and **ATTO 700** is quenched very efficiently by guanosine, tryptophan and related compounds. This process is based on electron transfer and requires direct contact between excited dye molecule and quenching agent.
2. The **ATTO-TEC** quenchers are designed to quench exclusively by the FRET mechanism. Thus, if there is no spectral overlap, no quenching takes place - in contrast to some other quenchers on the market!

Fluorescence Quenchers

ATTO 540Q

Optical properties of carboxy derivative

λ_{abs} = 543 nm

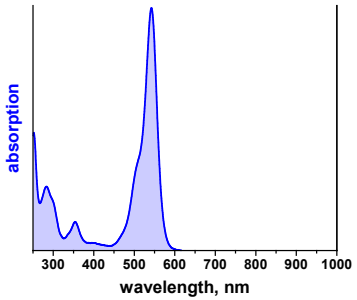
ε_{max} = 1.05 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.27

CF_{280} = 0.26

Features:

- High thermal and photo-stability
- Moderately hydrophilic
- Cationic rhodamine dye



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	659	559	AD 540Q-21	AD 540Q-25
NHS-ester	756	656	AD 540Q-31	AD 540Q-35
maleimide	781	681	AD 540Q-41	AD 540Q-45
streptavidin			AD 540Q-61	AD 540Q-65
biotin	970	869	AD 540Q-71	AD 540Q-75
phalloidin	1443	1329	AD 540Q-81*	AD 540Q-82**
azide	873	759	AD 540Q-101	AD 540Q-105

* 10 nmol **20 nmol

ATTO 575Q

Optical properties of carboxy derivative

λ_{abs} = 582 nm

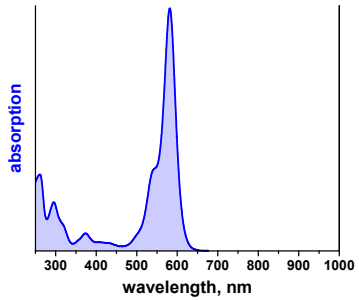
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.29

CF_{280} = 0.12

Features:

- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye related to rhodamines
- Single isomer



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	711	611	AD 575Q-21	AD 575Q-25
NHS-ester	808	708	AD 575Q-31	AD 575Q-35
maleimide	833	733	AD 575Q-41	AD 575Q-45

* 10 nmol **20 nmol

Fluorescence Quenchers

ATTO 580Q

Optical properties of carboxy derivative

λ_{abs} = 587 nm

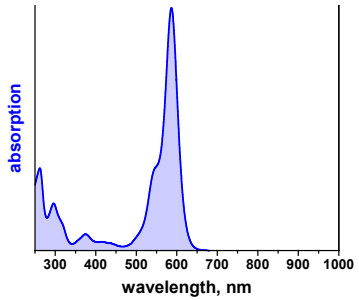
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.32

CF_{280} = 0.11

Features:

- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye related to rhodamines
- Supplied as mixture of six isomers



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	795	695	AD 580Q-21	AD 580Q-25
NHS-ester	892	792	AD 580Q-31	AD 580Q-35
maleimide	917	817	AD 580Q-41	AD 580Q-45
streptavidin			AD 580Q-61	AD 580Q-65
biotin	1106	1005	AD 580Q-71	AD 580Q-75
phalloidin	1579	1465	AD 580Q-81*	AD 580Q-82**

* 10 nmol **20 nmol

ATTO 612Q

Optical properties of carboxy derivative

λ_{abs} = 615 nm

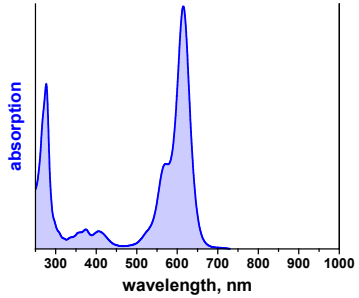
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.35

CF_{280} = 0.60

Features:

- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye related to rhodamines



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	791	691	AD 612Q-21	AD 612Q-25
NHS-ester	888	788	AD 612Q-31	AD 612Q-35
maleimide	913	813	AD 612Q-41	AD 612Q-45
streptavidin			AD 612Q-61	AD 612Q-65
biotin	1102	1001	AD 612Q-71	AD 612Q-75
phalloidin	1575	1461	AD 612Q-81*	AD 612Q-82**

* 10 nmol **20 nmol

Large Stokes-Shift Dyes

Dyes with Large Stokes-Shift

On excitation of a dye molecule a reorientation of the π-electron system takes place. This occurs extremely fast (faster than picoseconds). Due to the new charge distribution about the dye molecule the surrounding solvent molecules also move towards new equilibrium positions. As a consequence the energy of the entire system (excited dye molecule plus solvent) is lowered quickly, and the photons emitted have a lower energy than those needed for excitation. In other words: The fluorescence occurs at *longer* wavelengths than the excitation. The wavelength difference between fluorescence maximum and the corresponding absorption maximum is called *Stokes-shift*. With typical dyes the Stokes-shift amounts to 20 – 30 nm.

On excitation of dyes with highly *unsymmetrical* π-electron systems the dipole moment may change drastically. The ensuing strong reorientation of solvent molecules leads to an unusually large Stokes-shift, in particular in polar solvents like water and ethanol. As the non-radiative decay of the excited state is also enhanced by the solvent reorientation, the fluorescence quantum yield of such compounds is severely reduced in aqueous solutions. However, there are a few exceptions to this rule: Coumarin derivatives like **ATTO 390** and **ATTO 425** show a remarkably large Stokes-shift of about 90 and 50 nm, respectively, and yet fluoresce with a quantum yield of 90 % in water.

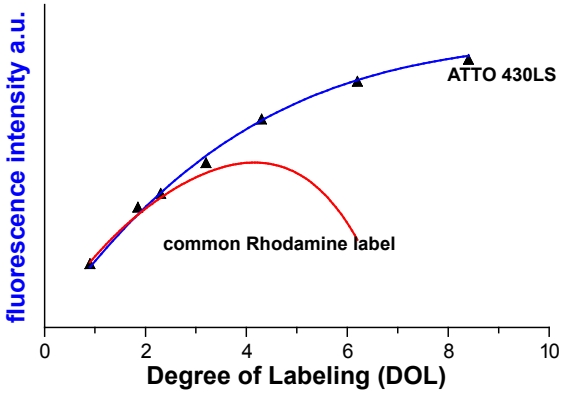
Even more remarkable is the dye **ATTO 465**. In spite of its symmetrical structure it has a large Stokes-shift of 55 nm in aqueous solution.

Optical Properties in PBS

Label	ε_{max}, $M^{-1}\,cm^{-1}$	λ_{abs}, nm	λ_{fl}, nm	Stokes-Shift, nm	η_{fl}, %	τ_{fl}, ns	Page
ATTO 390	24000	390	476	86	90	5.0	28
ATTO 425	45000	439	485	46	90	3.5	29
ATTO 465	75000	453	506	53	75	5.0	30

ATTO LS-Dye Series

ATTO-TEC's latest research led to new dyes featuring an extraordinary large Stokes-shift of up to 163 nm in aqueous solution (PBS). Thus the emission spectrum is almost completely separated from the absorption spectrum. The large fluorescence shift makes these dyes highly suitable for multicolor experiments. Fluorescence bleed-through between detection channels is minimized.
The dyes, **ATTO 430LS** and **ATTO 490LS**, are very hydrophilic and show excellent water solubility. They exhibit strong fluorescence. In contrast to many other commercial fluorophores, the fluorescence efficiency on conjugation to biomolecules (e.g. proteins) remains exceptionally high, even at a high degree of labeling (DOL).



Optical Properties in PBS

Label	ε_{max}, $M^{-1}\,cm^{-1}$	λ_{abs}, nm	λ_{fl}, nm	Stokes-Shift, nm	η_{fl}, %	τ_{fl}, ns	Page
ATTO 430LS	32000	436	545	109	65	4.0	72
ATTO 490LS	40000	495	658	163	30	2.6	73

ATTO 430LS

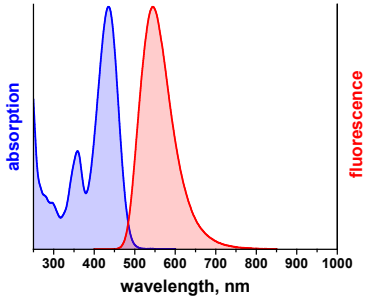
Optical properties of carboxy derivative

λ_{abs} = 436 nm
ε_{max} = 3.2 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 545 nm
η_{fl} = 65 %
τ_{fl} = 4.0 ns

CF_{260} = 0.32
CF_{280} = 0.22

Features:

- Extraordinary large Stokes-shift
- High fluorescence yield
- Highly fluorescent even after conjugation
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy



Modification	MW, g/mol	MH^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	589	567	AD 430LS-21	AD 430LS-25
NHS-ester	686	664	AD 430LS-31	AD 430LS-35
maleimide	711	689	AD 430LS-41	AD 430LS-45
streptavidin			AD 430LS-61	AD 430LS-65
phalloidin	1359	1337	AD 430LS-81*	AD 430LS-82**
azide	789	767	AD 430LS-101	AD 430LS-105

* 10 nmol **20 nmol

Large Stokes-Shift Dyes

ATTO 490LS

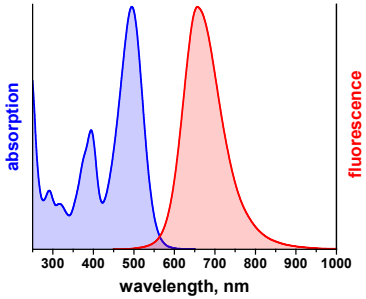
Optical properties of carboxy derivative

λ_{abs} = 495 nm
ε_{max} = 4.0 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 658 nm
η_{fl} = 30 %
τ_{fl} = 2.6 ns

CF_{260} = 0.39
CF_{280} = 0.21

Features:

- Extraordinary large Stokes-shift
- High fluorescence yield
- Highly fluorescent even after conjugation
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
carboxy	696	674	AD 490LS-21	AD 490LS-25
NHS-ester	793	771	AD 490LS-31	AD 490LS-35
maleimide	818	796	AD 490LS-41	AD 490LS-45
streptavidin			AD 490LS-61	AD 490LS-65
phalloidin	1466	1444	AD 490LS-81*	AD 490LS-82**
azide	896	874	AD 490LS-101	AD 490LS-105

* 10 nmol **20 nmol

Customized Labels and Products

In addition to the products described in this catalogue **ATTO-TEC** offers on request dyes and labels taylored to the special needs of its customers.
The following examples may illustrate the possibilities.

Derivatives of ATTO-Labels

Linker

In most ATTO-labels the reactive group (NHS-ester etc.) is connected with the fluorophore by a linker consisting of a 4-atom flexible chain. For most applications this has proven to be very suitable and practical. However, we can provide ATTO dyes with linker that differ in:

- **flexibility**
- **length** (e.g. cadaverine derivatives of ATTO 488, ATTO 532, ATTO 550, ATTO 647**N**, and ATTO 680, ATTO 590-ethylenediamine, ATTO 647**N**-PEG-(12)-NHS, ATTO 633-PEG-(8)-azide, ATTO 633-PEG-(12)-azide, ATTO 740-PEG-(8)-azide, ATTO 647**N**-aminohexylcarboxylic acid, ATTO 532-aminohexylcarboxylic acid, ATTO 565-propylazide ...
- **rigidity**
- **...**

Reactive Group and Conjugates

N-hydroxysuccinimidyl(NHS)-ester and maleimide are the most common reactive groups for coupling to amine and thiol, respectively. However, for other substrate functionalities it is necessary that the labels carry an entirely different reactive group: **ATTO-TEC** accordingly provides a variety of reactive groups and conjugates:

- AEDP-NHS (e.g. ATTO 488 ...)
- alkyne
- amine (cadaverine)
- cycloalkyne (e.g. DBCO)
- hydrazide (e.g. ATTO 488, ATTO 514, ATTO 532, ATTO 550, ATTO 565, ATTO 590, ATTO 633, ATTO 647**N** ...)
- lipids: DSPE, DLPE (e.g. ATTO 488, ATTO 647**N** ...)
- sulfo-NHS (e.g. ATTO 610-sulfo-NHS ...)
- tetrazine
- ...

If a particular ATTO-dye derivative or conjugate is not listed in this catalogue, it might still be available on request. Send your inquiry to info@atto-tec.com.

Solubility, Charges

On customer request ATTO-dyes can be rendered very hydrophobic or else very hydrophilic and thus become compatible with a particular solvent, surface, or biochemical environment. Furthermore the electrical charge of a label can be adapted to achieve the desired interaction with a biomolecule or simply to obtain a special migration behaviour in electrophoresis.

Special Dyes

New Chromophores

Most labels described in this catalogue are based on dyes patented by **ATTO-TEC**. These products have been selected for optimum value to our customers, and we at **ATTO-TEC** are committed to provide these dyes and their derivatives for years to come. However, continuous scientific progress and the invention of ever new applications require the development of novel dyes and derivatives. Therefore, if you need a fluorescent label with special properties, not found in the catalogue, please let us know. We will try to help.

Recommended Procedures for Labeling

Introduction

ATTO-TEC offers a large variety of high-quality dyes for labeling amino and thiol groups. ATTO reactive dyes cover the spectral region from 350 nm in the UV to 750 nm in the NIR.
The most commonly used amine-reactive dye derivatives are N-hydroxysuccinimidyl(NHS)-esters. NHS-esters readily react with amine-modified oligonucleotides or amino groups of proteins, i.e. the ε-amino groups of lysines or the amine terminus, forming a chemically stable amide bond between the dye and the protein or oligo. However, the amino group ought to be unprotonated to be reactive. Therefore the pH of the solution must be adjusted sufficiently high to obtain a high concentration of unprotonated amino groups. On the other hand, the NHS-ester also reacts with the hydroxyl ions in the solution to yield free dye, which is no longer reactive. As the rate of this hydrolysis increases with the concentration of hydroxyl ions, the pH should be kept as low as possible. Buffering the solution at pH 8.3 has been found to be a good compromise between the contradicting requirements.
For labeling thiol groups the most popular and commonly used dye derivatives are maleimides and iodoacetamides. They react with thiol groups of proteins to form a stable thio-ether bond.

Labeling Proteins with Amine-Reactive ATTO-Labels

ATTO NHS-esters readily react with amino groups of proteins. The optimum pH range for NHS-ester coupling is pH 8.0 – 9.0. At this pH amino groups of proteins, i.e. the ε-amino groups of lysines are unprotonated to a high degree and highly reactive towards the dye-NHS-ester.

Labeling Procedures

Required Materials

- **Solution A**: PBS buffer (Phosphate-Buffered Saline, pH 7.4): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4 \cdot 2\ H_2O$, and 0.24 g KH_2PO_4, in 1 liter distilled water.
- **Solution B**: 0.2 M sodium bicarbonate solution, adjusted to pH 9.0 with 2 M sodium hydroxide.
- **Solution C**: To 20 parts of **Solution A** add 1 part of **Solution B** to obtain a labeling buffer of pH 8.3. Kept in an air-tight bottle, this solution will be stable for a long period of time.
- **Solution D**: Dissolve 1.0 mg of dye NHS-ester in 50 – 200 µl of anhydrous, amine-free DMSO or acetonitrile. Depending on solvent quality such solutions are not stable at room temperature and for storage purposes must be kept, protected from light, at -20 °C. Additionally, it may be difficult to avoid humidity entering a solution in continuous use. In the presence of water NHS-esters readily hydrolyze and become non-reactive. We advise to freshly prepare, whenever possible, the dye NHS-ester solution immediately before starting the labeling reaction.
- Gel filtration column filled with Sephadex G-25 or equivalent.

Conjugate Preparation

- Dissolve 1 – 5 mg of protein in 1 ml of **Solution C**. Protein solutions must be free of any amine-containing substances such as tris-(hydroxymethyl)-aminomethane (TRIS), free amino acids or ammonium ions. Antibodies that are dissolved in amine containing buffers should be dialyzed against **Solution A**, and the desired coupling pH of 8.3 will be obtained by the procedure given above for **Solution C**. The presence of sodium azide in low concentration (< 3 mM) will not interfere with the labeling reaction.
- To obtain a degree of labeling (DOL, dye-to-protein ratio) of 2 – 3 add, while gently shaking, a threefold molar excess of reactive dye (**Solution D**) to the protein solution. Variations due to different reactivities of both the protein and the labeling reagent may occur. This may necessitate optimization of the dye-to-protein ratio used in the reaction in order to obtain the desired DOL. To increase the degree of labeling a higher ratio of NHS-ester to protein has to be used and vice versa.

- Incubate the reaction mixture protected from light for up to 1 hour at room temperature. In most cases the labeling reaction will be complete within 5 – 10 minutes.

Conjugate Purification – Removal of Unbound Dye

- Due to an unavoidable side reaction part of the applied dye NHS-ester will hydrolyze during the labeling reaction and must be removed via gel filtration using Sephadex G-25 or equivalent. We recommend a column with 1 – 2 cm in diameter and 15 – 20 cm in length. For very hydrophilic dyes, e. g. ATTO 488, ATTO 532, ATTO 542, ATTO 594, the column has to be at least 30 cm in length to achieve a satisfactory result.
- Preequilibrate the column with **Solution A**.
- Elute the dye-protein conjugate using **Solution A**.
- The first colored and fluorescent zone to elute will be the desired dye-protein conjugate. A second colored and fluorescent, but slower moving zone contains the unbound free dye (hydrolyzed NHS-ester).
- To prevent denaturation of the conjugate after elution, bovine serum albumin (BSA) or another stabilizer may be added.
- For re-use of the Sephadex column one can elute with either 0.01 % sodiumhydroxide solution and/or water/ethanol 80:20 to remove any residues of dye or dye-conjugate. The treatment is followed by exhaustive washing with water.

Labeling Proteins with Thiol-Reactive ATTO-Labels

ATTO maleimides (MAL) and iodoacetamides (IAA) readily react with thiol groups of proteins. The optimum acidity for thiol modification is pH 7.0 – 7.5 in the case of maleimides and pH 8.0 – 8.5 for the lesser reactive iodoacetamides. At these pH the thiol (sulfhydryl) group is deprotonated to a sufficient degree and readily reacts with the dye-maleimide or dye-iodoacetamide.

Required Materials

- **Solution A**: PBS buffer (Phosphate-Buffered Saline, pH 7.4): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4 \cdot 2\ H_2O$, and 0.24 g KH_2PO_4, in 1 liter distilled water.
- **Solution B**: 0.2 M sodium bicarbonate solution, adjusted to pH 9.0 with 2 M sodium hydroxide.
- **Solution C**: To 20 parts of **Solution A** add 1 part of **Solution B** to obtain a labeling buffer of pH 8.3. Kept in an air-tight bottle, this solution will be stable for a long period of time.
- **Solution E**: Dissolve 1.0 mg of dye-maleimide in 50 – 200 µl of anhydrous, amine-free DMF or acetonitrile. Depending on solvent quality such solutions are not stable at room temperature and for storage purposes must be kept, protected from light, at -20 °C. Additionally, it may be difficult to avoid humidity entering a solution in continuous use. The maleimide moiety may hydrolyze and become non-reactive. We advise to freshly prepare, whenever possible, the dye-maleimide solution immediately before starting the labeling reaction.
- **Solution F**: Dissolve 1.0 mg of dye-iodoacetamide in 50 – 200 µl of anhydrous, amine-free DMF or acetonitrile. Depending on solvent quality such solutions are not stable at room temperature and for storage purposes must be kept, protected from light, at -20 °C. We strongly recommend to freshly prepare, whenever possible, the dye-iodoacetamide solution immediately before starting the labeling reaction.
- Gel filtration column filled with Sephadex G-25 or equivalent.

Conjugate Preparation

A. Maleimide Conjugation

- Dissolve 1 – 5 mg of protein in 1 ml of **Solution A** (PBS buffer, pH 7.4).
- The free thiol group will react with dye-maleimide by adding a 1.3 fold molar excess of reactive dye (**Solution E**) while gently shaking. Variations due to different reactivities of both the protein and the labeling reagent may occur.
- Incubate the reaction mixture protected from light for 2 hours at room temperature.

B. Iodoacetamide Conjugation

- Dissolve 1 – 5 mg of protein in 1 ml of **Solution C** (PBS buffer, pH 8.3).
- The free thiol group will react with 1.3 fold molar excess of reactive dye (**Solution F**) while gently shaking. Incubate the reaction mixture, protected from light for 2 hours at **37 °C**. The slight rise in temperature speeds up the conjugation reaction drastically. At room temperature it may take more than 10 hours to complete conjugation.

Note: While dye-maleimides and iodoacetamides react readily with thiol (mercapto or sulfhydryl) groups, there is absolutely no reaction with disulfides. If the protein contains disulfide bonds and labeling at their position is desired, it is necessary to reduce the disulfide to thiol groups before labeling. For reduction, reagents such as tris(2-carboxyethyl)phosphin (TCEP) or dithiothreitol (DTT) may be used. However, great care has to be taken that any excess of these reducing agents has been removed (e.g. by dialysis) as they consume dye-maleimide themselves and in some cases (ATTO 725, ATTO 740, ATTO 610, ATTO 647) even destroy the dye chromophore.

Conjugate Purification – Removal of Unbound Dye

- Excess and hydrolyzed dye must be removed from the protein conjugate via gel filtration using Sephadex G-25 or equivalent. We recommend a column with 1 – 2 cm in diameter and 15 – 20 cm in length. For very hydrophilic dyes, e. g. ATTO 488, ATTO 532, ATTO 542, ATTO 594, the column has to be at least 30 cm in length to achieve a satisfactory result.
- Preequilibrate the column with **Solution A**.
- Elute the dye-protein conjugate using **Solution A**.
- The first colored and fluorescent zone to elute will be the desired dye-protein conjugate. A second colored and fluorescent, but slower moving zone contains the unbound free or hydrolyzed dye
- To prevent denaturation of the conjugate after elution, bovine serum albumin (BSA) or another stabilizer may be added.
- For re-use of the Sephadex column one can elute with either 0.01 % sodiumhydroxide solution and/or water/ethanol 80:20 to remove any residues of dye or dye-conjugate. The treatment is followed by exhaustive washing with water.

Labeling Procedures

Storage of the Protein Conjugates

In general, conjugates should be stored under the same conditions used for the unlabeled protein. For storage in solution at 4 °C, sodium azide (2 mM final concentration) can be added as a preservative. Removal of preservatives prior to use may be necessary to avoid inhibitory effects in applications in which conjugates are added to live cell specimens. The conjugate should be stable at 4 °C for several months. For long-term storage, divide the solution into small aliquots and freeze at -20 °C. Avoid repeated freezing and thawing. Protect dye conjugates from light as much as possible.

Determining the Degree of Labeling (DOL)

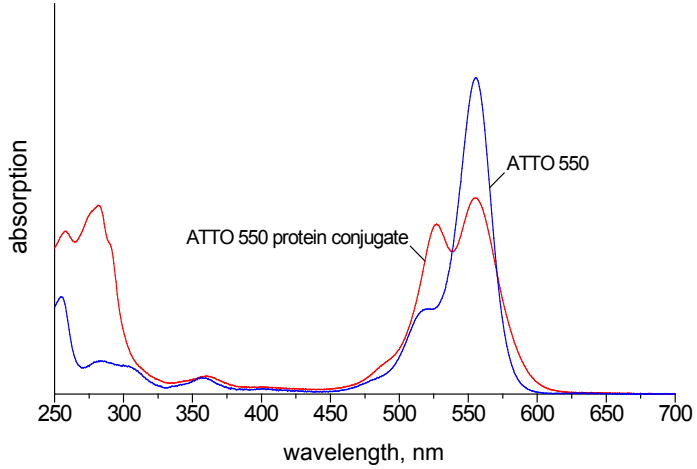
The degree of labeling (DOL, dye-to-protein ratio) obtained by the above procedures can be determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ε) × molar concentration (c) × path length (d). Simply measure the UV-VIS spectrum of the conjugate solution as obtained after gel filtration in a quartz (UV-transparent) cell. You may need to dilute the solution, if it turns out to be too concentrated for a correct absorbance measurement. Determine the absorbance (A_{max}) at the absorption maximum (λ_{abs}) of the dye and the absorbance (A_{280}) at 280 nm (absorption maximum of proteins). The concentration of bound dye is given by: $c(dye) = A_{max} / \varepsilon_{max} \times d$, where ε_{max} is the extinction coefficient of the dye at the absorption maximum. The protein concentration is obtained in the same way from its absorbance at 280 nm. As all dyes show some absorption at 280 nm, the measured absorbance A_{280} must be corrected for the contribution of the dye. This is given by $A_{max} \times CF_{280}$. The values for the correction factor $CF_{280} = \varepsilon_{280} / \varepsilon_{max}$ are listed in the table on p. 84. It follows for the absorbance of the protein itself: $A_{prot} = A_{280} - A_{max} \times CF_{280}$. Then the concentration of protein is:
$c(protein) = A_{prot} / \varepsilon_{prot} \times d$, where ε_{prot} is the extinction coefficient of the protein at 280 nm.

Labeling Procedures

It follows for the degree of labeling, i.e. the average number of dye molecules coupled to a protein molecule: DOL = c(dye) / c(protein) and with the above relations:

$$DOL = \frac{A_{max} / \varepsilon_{max}}{A_{prot} / \varepsilon_{prot}} = \frac{A_{max} \cdot \varepsilon_{prot}}{(A_{280} - A_{max} \cdot CF_{280}) \cdot \varepsilon_{max}}$$

Note: The above equation is only valid if the extinction coefficient ε_{max} of the free dye at the absorption maximum is the same as the extinction coefficient of the conjugated dye at this wavelength. Due to dye aggregation effects this is frequently not the case. Hence the value calculated for DOL may be too low by 20 % or more. This is illustrated by direct comparison of the absorption spectra of ATTO 550 as free, i.e. unbound, dye (blue curve) and the same amount of dye, conjugated to a protein (red curve).



Although ATTO-dye molecules are small compared to biomolecules like proteins, DNA etc., they will affect their properties to a certain degree. Notably mass and, frequently, electrical charge of the biomolecule will be different after conjugation with a dye. To aid in the **analysis of biomolecule-dye conjugates**, the table on page 84-85 shows the increase in mass (Δm) and charge (Δq) that occur on coupling with an ATTO-dye. Because biomolecules as well as ATTO-dyes may carry basic (-NH_2) and acidic (-COOH, -SO_3H) substituents, both mass and electrical charge depend on pH. The data given in the table are based on the assumption of non-protonated amino groups (-NH_2), deprotonated acid groups (-COO^-, -SO_3^-) and neutral thiol groups. This reflects the situation given in a close-to-neutral environment (pH 6 – 8). It is worth mentioning that under more acidic conditions (pH < 4) the additional, non-reactive, carboxylic acid group of dyes like ATTO 565 and ATTO 590 will be protonated. As a consequence both mass and charge will be higher by one unit than the values given in the table, which are valid for pH 6 – 8.

For the preparation of dye stock-solutions a solvent recommendation for each dye is given in the table on page 84-85. One should keep in mind that solvents like DMSO or DMF are never free of nucleophilic and/or basic impurities. Such compounds will react with the NHS-ester, maleimide and iodoacetamide functionality and consequently reduce coupling efficiency. In some cases (ATTO 610, ATTO 647, ATTO 725, ATTO 740) they also undergo reactions with the dye chromophore resulting in dye-degradation.

Labeling Procedures

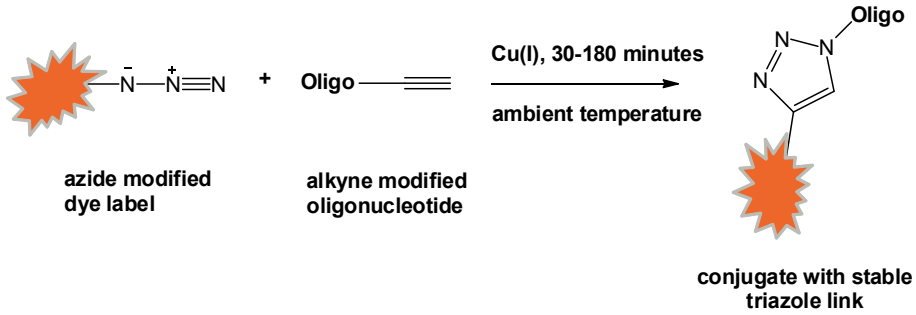
Dye	MW, g/mol NHS	MAL	IAA	Δm, g/mol NHS	MAL	IAA	Δq
ATTO 390	440	466	553	325.4	465.5	425.5	0
ATTO 425	499	524		383.4	523.6		0
ATTO 430LS	686	711		547.7	687.8		-1
ATTO 465	493	518		278.4	418.5		+1
ATTO 488	981	1067	914	570.6	710.7	670.7	-1
ATTO 490LS	793	818		654.8	795.0		-1
ATTO 495	549	574		334.4	474.6		+1
ATTO 514	1111	990	1078	734.6	874.7	834.8	-1
ATTO 520	564	589		349.5	489.6		+1
ATTO 532	1081	1063	970	627.7	766.8	726.8	-1
ATTO Rho6G	711	750		496.6	636.7		+1
ATTO 540Q	756	781		541.6	541.6		+1
ATTO 542	1125	1150		893.0	1033.1		-3
ATTO 550	791	816	980	576.8	716.9	678.9	+1
ATTO 565	708	733	835	492.2	632.7	593.7	+1
ATTO Rho3B	739	764		524.7	664.8		+1
ATTO Rho11	763	788		548.7	688.8		+1
ATTO Rho12	847	872		632.9	773.0		+1
ATTO Thio12	699	724		484.6	624.8		+1
ATTO Rho101	787	812		572.7	712.9		+1
ATTO 575Q	808	833		591.7	733.8		+1
ATTO 580Q	892	917		677.9	818.0		+1
ATTO 590	788	813	931	572.7	712.8	673.8	+1
ATTO 594	1389	1358	1129	786.9	927.1	831.9	-1
ATTO Rho13	843	868		628.8	769.0		+1
ATTO 610	588	613		373.5	513.7		+1
ATTO 612Q	888	913		673.8	814.0		+1
ATTO 620	709	734		494.7	634.8		+1
ATTO Rho14	981	1020		766.6	906.8		+1
ATTO 633	749	774	876	534.7	674.9	634.8	+1
ATTO 647	811	829		574.8	714.9		0
ATTO 647**N**	843	868	970	628.9	769.0	729.0	+1
ATTO 655	887	812	852	509.6	649.8	610.8	0
ATTO Oxa12	835	875		621.9	762.0		+1
ATTO 665	820	845		605.7	745.9		+1
ATTO 680	828	1024	850	507.6	647.8	608.7	0
ATTO 700	837	971		547.7	687.8		0
ATTO 725	613	638		398.5	538.7		+1
ATTO 740	665	690		450.6	590.8		+1
ATTO MB2	553	591		338.4	478.5		+1

λ_{abs}, nm	ε_{max}, M^{-1} cm^{-1}	λ_{fl}, nm	CF_{260}	CF_{280}	Recommended Solvent NHS	MAL/IAA
390	2.4×10^4	476	0.46	0.09	DMSO	DMF
439	4.5×10^4	485	0.19	0.17	DMSO	DMF
436	3.2×10^4	545	0.32	0.22	DMSO	DMF
453	7.5×10^4	506	1.09	0.48	DMSO	DMF
500	9.0×10^4	520	0.22	0.09	DMSO	DMF
495	4.0×10^4	658	0.39	0.21	DMSO	DMF
498	8.0×10^4	526	0.45	0.37	DMSO	DMF
511	1.15×10^5	532	0.21	0.07	DMSO	DMF
517	1.1×10^5	538	0.16	0.20	DMSO	DMF
532	1.15×10^5	552	0.20	0.09	DMSO	DMF
533	1.15×10^5	557	0.19	0.16	DMSO	DMF
543	1.05×10^5		0.27	0.26	DMSO	DMF
542	1.2×10^5	562	0.18	0.08	DMSO	DMF
554	1.2×10^5	576	0.23	0.10	DMSO	DMF
564	1.2×10^5	590	0.27	0.12	DMSO	DMF
566	1.2×10^5	589	0.27	0.13	DMSO	DMF
572	1.2×10^5	595	0.26	0.10	DMSO	DMF
577	1.2×10^5	600	0.26	0.09	DMSO	DMF
582	1.1×10^5	607	0.11	0.37	DMSO	DMF
587	1.2×10^5	609	0.18	0.17	DMSO	DMF
582	1.2×10^5		0.29	0.12	DMSO	DMF
587	1.1×10^5		0.32	0.11	DMSO	DMF
593	1.2×10^5	622	0.39	0.43	DMSO	DMF
603	1.2×10^5	626	0.22	0.50	DMSO	DMF
603	1.2×10^5	627	0.28	0.43	DMSO	DMF
616	1.5×10^5	633	0.03	0.06	ACN	ACN
615	1.15×10^5		0.35	0.60	DMSO	DMF
620	1.2×10^5	642	0.04	0.06	DMSO	DMF
626	1.4×10^5	646	0.26	0.47	DMSO	DMF
630	1.3×10^5	651	0.04	0.06	DMSO	DMF
647	1.2×10^5	667	0.08	0.04	ACN	ACN
646	1.5×10^5	664	0.04	0.03	DMSO	DMF
663	1.25×10^5	680	0.24	0.08	DMSO	DMF
662	1.25×10^5	681	0.32	0.12	DMSO	DMF
662	1.60×10^5	680	0.07	0.06	DMSO	DMF
681	1.25×10^5	698	0.30	0.17	DMSO	DMF
700	1.2×10^5	716	0.26	0.41	DMSO	DMF
728	1.2×10^5	751	0.08	0.06	ACN	ACN
743	1.2×10^5	763	0.07	0.07	ACN	ACN
668	1.0×10^5		0.08	0.24	DMSO	DMF

ATTO-Reagents for Click Chemistry

Introduction

ATTO-TEC offers a large variety of high-quality reagents for Click Chemistry. The term “Click Chemistry” describes chemical reactions that are able to quickly and reliably generate substances by joining together small units. One of the most popular reactions within the Click Chemistry concept is the copper (I) catalyzed Huisgen azide alkyne cycloaddition forming a covalent linking unit (triazole) between the label and the target molecule.



Protocol for Oligonucleotide Labeling via Click Chemistry

General Remarks

The following protocol describes the labeling procedure for 10 nmol of a single alkyne modified oligonucleotide.
The reaction is most efficient if the azide and alkyne are dissolved in a minimal amount of solvent and the solutions are of high concentration.
The reaction can be accelerated by raising the temperature and is generally finished in 30 min at around 40 – 45 °C.

Required Materials

- **Solution A**: Dissolve the azide or alkyne modified oligonucleotide in the appropriate amount of water to obtain a 2 mM solution and centrifuge shortly.
- **Solution B**: Dissolve 1.0 mg of the click reagent (azide or alkyne modified ATTO-dye) in the appropriate amount of DMSO/t-BuOH-solution 1:1 to obtain a 50 mM solution.

Labeling Procedures

- **Solution C**: Click Solution: Dissolve 54 mg TBTA in 1 ml DMSO/t-BuOH 3:1 for a 0.1 M solution. The solution can be stored at -20 °C.
- **Solution D**: Dissolve 1 mg CuBr in 70 µl DMSO/t-BuOH 3:1 to obtain a 0.1 M solution.
  NOTE: This solution must be freshly prepared and cannot be stored!
- **Solution E**: The final click solution is prepared by quickly adding 1 volume **Solution D** to 2 volumes of **Solution C**.

Conjugate Preparation

In general the labeling reaction works more efficiently with concentrated solutions of alkynes (e.g. oligo) and azides (dye label). In the case the reaction does not work in water, rising the pH by performing the reaction in Tris-HCl (50 mM) at pH 8.3 might be helpful.

- Pipette 5 µl of **Solution A** (10 nmol of oligonucleotide) in a 0.5 ml reaction vial.
- Add 1 – 2 µl of **Solution B** (50 – 100 nmol; 5 – 10 eq.) to the reaction vial.
- 3 µl of freshly prepared **Solution E** is added and the reaction vial is thoroughly mixed by shaking at 25 °C for 3 h. As previously mentioned, by rising the temperature to 40 – 45 °C the reaction is generally finished in 30 minutes.

Conjugate Purification – Removal of Excess Reagent

- The reaction is subsequently diluted with 100 µl of 0.3 M NaOAc solution and the oligo precipitated by adding 1 ml cold absolute ethanol. The supernatant is removed and the residue washed twice with 100 µl cold ethanol. The washed residue is redissolved in pure water (20 µl) and can be used without further purification.
- Alternatively centrifuge for 10 min, remove the supernatant and dry the residue on air.

Storage of the Labeled Oligo

In general, conjugates should be stored under the same conditions used for the unlabeled oligonucleotide. For long term storage we recommend to freeze at -20 °C.

Labeled Phospholipids

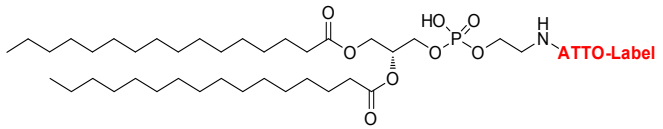
Fluorescence Labeled Membrane Probes

The investigation of biological membranes, e.g. intracellular membranes of live cells, plasma membranes etc., has become a major area of interest. As a result there is a growing demand for fluorescent lipids, in particular phospholipids to be incorporated in biological membranes. **ATTO-TEC** now offers a variety of phospholipids based on glycerol carrying one or two fatty acids (lipophilic groups) and a phosphate monoester residue (hydrophilic group).
Natural phospholipids are the predominant building blocks of biological membranes and are generally very similar in structure. However, minor differences, e.g. number and length of the fatty acid chains, degree of unsaturation of the fatty acid and nature of hydrophilic head group may result in significant variations of the physical properties and biological activity of such membranes.
ATTO-fluorescent phospholipids are labeled at the hydrophilic head group. After incorporation of the fluorescent phospholipid the fluorophore is located at the water/lipid interface of the membrane. Currently we provide **1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine** (DPPE), **1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine** (DOPE), **1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine** (PPE), and **1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine** (DMPE) labeled with selected ATTO-dyes. We label other ATTO-dyes on request.
Also for other labeled phospholipids such as 1,2 distereoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) or 1,2 dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) please send an e-mail to info@atto-tec.com

Storage and Handling:

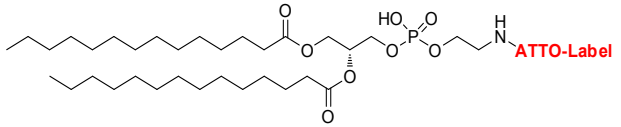
Fluorescent phospholipid derivatives are supplied in solid form and should be stored at ≤ -20 °C, desiccated and protected from light. When stored as indicated, ATTO-dye labeled phospholipids are stable for at least three years.
For the preparation of stock solutions we recommend using chloroform/methanol 80:20 as solvent of choice. The stock solution of labeled phospholipids should be stored in the same way as the solid, however the shelf life of such solutions might be significantly reduced.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE)



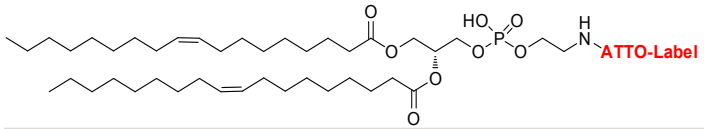
	MW, g/mol	Order Code 1 mg	5 mg
ATTO 390 DPPE	1017	AD 390-151	AD 390-155
ATTO 488 DPPE	1264	AD 488-151	AD 488-155
ATTO 520 DPPE	1040	AD 520-151	AD 520-155
ATTO 532 DPPE	1320	AD 532-151	AD 532-155
ATTO 542 DOPE *new*	1588	AD 542-151	AD 542-155
ATTO 550 DPPE	1382	AD 550-151	AD 550-155
ATTO 565 DPPE	1299	AD 565-151	AD 565-155
ATTO 590 DPPE	1379	AD 590-151	AD 590-155
ATTO 594 DPPE	1480	AD 594-151	AD 594-155
ATTO 633 DPPE	1326	AD 633-151	AD 633-155
ATTO 647**N** DPPE	1420	AD 647**N**-151	AD 647**N**-155
ATTO 655 DPPE *new*	1316	AD 655-151	AD 655-155
ATTO 700 DPPE	1353	AD 700-151	AD 700-155
ATTO 740 DPPE	1142	AD 740-151	AD 740-155

1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE)



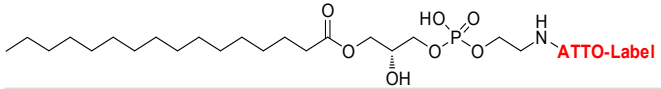
	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 DMPE	1207	AD 488-191	AD 488-195
ATTO 520 DMPE	984	AD 520-191	AD 520-195
ATTO 532 DMPE	1264	AD 532-191	AD 532-195
ATTO Rho6G DMPE	1232	AD Rho6G-191	AD Rho6G-195
ATTO 550 DMPE	1312	AD 550-191	AD 550-195
ATTO 590 DMPE	1309	AD 590-191	AD 590-195
ATTO 594 DMPE	1424	AD 594-191	AD 594-195
ATTO 633 DMPE	1270	AD 633-191	AD 633-195
ATTO 647**N** DMPE	1364	AD 647**N**-191	AD 647**N**-195

1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)



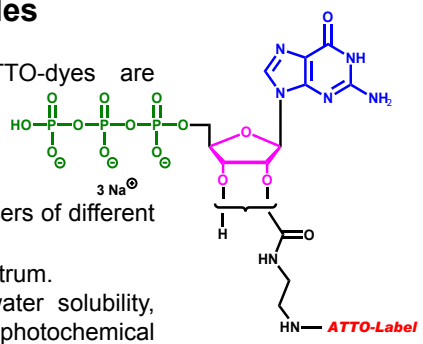
	MW, g/mol	Order Code 1 mg	5 mg
ATTO 390 DOPE *new*	1069	AD 390-161	AD 390-165
ATTO 488 DOPE	1316	AD 488-161	AD 488-165
ATTO 520 DOPE	1092	AD 520-161	AD 520-165
ATTO 532 DOPE	1372	AD 532-161	AD 532-165
ATTO 540Q DOPE *new*	1285	AD 540Q-161	AD 540Q-165
ATTO 542 DOPE *new*	1640	AD 542-161	AD 542-165
ATTO 550 DOPE	1420	AD 550-161	AD 550-165
ATTO 590 DOPE	1417	AD 590-161	AD 590-165
ATTO 594 DOPE	1532	AD 594-161	AD 594-165
ATTO 633 DOPE	1378	AD 633-161	AD 633-165
ATTO 647 DOPE *new*	1319	AD 647-161	AD 647-165
ATTO 647**N** DOPE	1485	AD 647**N**-161	AD 647**N**-165
ATTO 655 DOPE	1368	AD 655-161	AD 655-165
ATTO 665 DOPE *new*	1349	AD 665-161	AD 665-165
ATTO 680 DOPE	1366	AD 680-161	AD 680-165
ATTO 740 DOPE	1194	AD 740-161	AD 740-165

1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (PPE)



	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 PPE	1025	AD 488-181	AD 488-185
ATTO 520 PPE	802	AD 520-181	AD 520-185
ATTO 532 PPE	1081	AD 532-181	AD 532-185
ATTO 550 PPE	1130	AD 550-181	AD 550-185
ATTO 590 PPE	1127	AD 590-181	AD 590-185
ATTO 594 PPE	1241	AD 594-181	AD 594-185
ATTO 633 PPE	1088	AD 633-181	AD 633-185
ATTO 647**N** PPE	1194	AD 647**N**-181	AD 647**N**-185

Fluorescence-Labeled Nucleotides



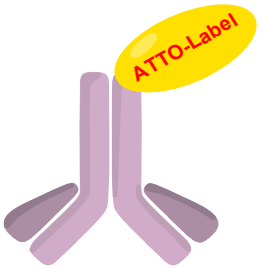
Fluorescence-labeled nucleotides with ATTO-dyes are available from Jena Bioscience.

Features:

- Labeling at different positions with spacers of different lengths.
- Labels that cover the entire visible spectrum.
- Extraordinary properties (e.g. good water solubility, high signal intensity, chemical and photochemical stability).

All labeled nucleotides are supplied as ready-to-use aqueous solutions in various units and concentrations depending on the particular nucleotide and/or label.
For detailed information please visit www.jenabioscience.com.

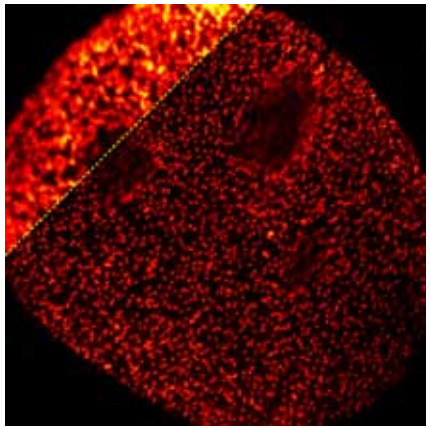
Fluorescence-Labeled Antibodies



ATTO-labeled antibodies are available from several renowned companies. They are fully licensed to offer their antibodies with numerous **ATTO**-dyes. A list of suppliers can be found on our website at www.atto-tec.com under “Miscellaneous - Labeled Antibodies“.

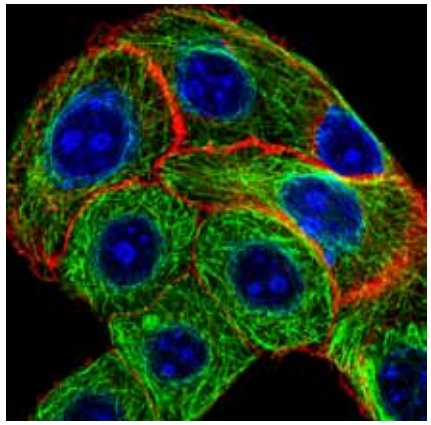
Fluorescence-Labeled Oligonucleotides

Oligonucleotides are widely used in methods such as DNA-sequencing, polymerase chain reaction (PCR), genetic and forensic analysis and many more. Due to its high sensitivity in most of these applications the method of detection is based on fluorescence. Consequently oligonucleotides need to be labeled with a fluorophore for visualization.
ATTO-labeled oligonucleotides are provided by many companies specialized in oligonucleotide synthesis. A full list of suppliers can be found on our website at www.atto-tec.com under "*Miscellaneous - Labeled Oligonucleotides*".

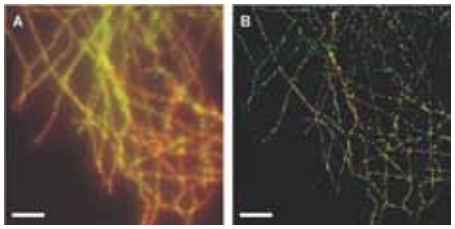


GM5756T - nuclear pore labeled with **ATTO 490LS**.

A single living U2OS cell was labeled with three different fluorescent probes delivered from a single barrel nanopipette. The labeled cellular structures were visualized by 3D fluorescence imaging. Actin was visualized by **ATTO 655−phalloidin** (red), β-tubulin by paclitaxel−Oregon Green (green), and the nucleus was stained with DAPI (blue).
Nano Lett. 2015, 15, 1374−1381

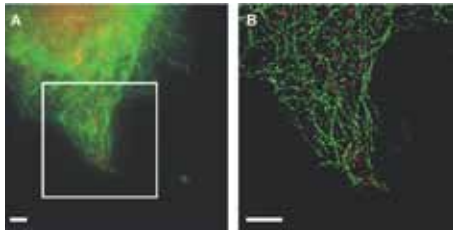


Methanol fixed MDA-MB-468 cells; EGF-receptor stained with **AZ 271** (ATTO-TEC) labeled affibody (red), Ki67 stained with rabbit IgG anti Ki67 (primary) and **ATTO 647N** labeled donkey IgG-fab2-fragment anti rabbit (secondary) (blue); tubulin-antibody staining with mouse IgG anti beta tubulin (primary) and donkey IgG-fab2-fragment anti mouse labeled with **ATTO 488** (secondary) (green).

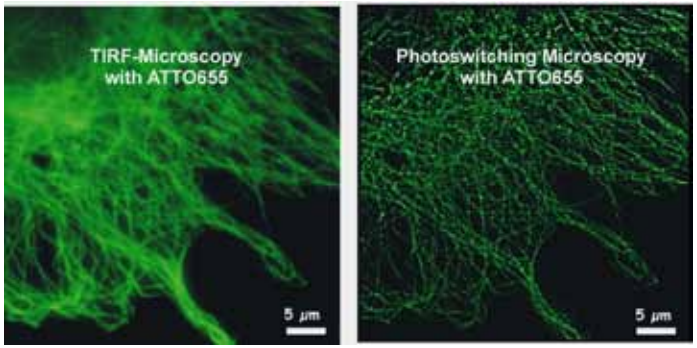


Dual-color photoswitching microscopy with **ATTO 520** and **ATTO 655** labeled microtubules in COS-7 cells. The reconstructed photoswitching image is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed sequentially in PBS, pH 7.4 in the presence of 100 mM mercapto ethylamine at laser powers of 30 mW at 647 nm and 514 nm with a frame rate of 20 Hz. 16000 images were taken from the two spectrally different fluorophores (scale bar 5 µm).

M. Sauer et al., University of Würzburg.

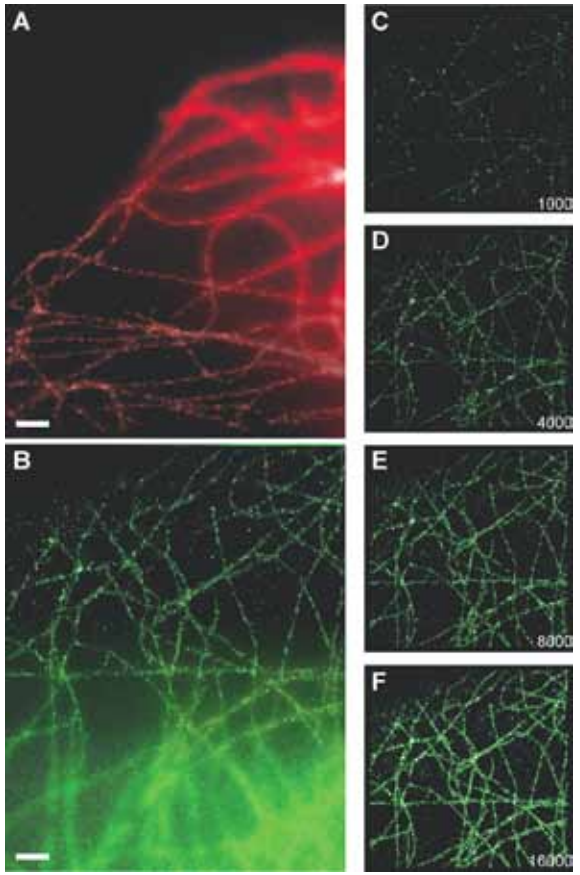


Dual-color photoswitching microscopy with **ATTO 520** labeled microtubules and **ATTO 655** labeled cytochrome c oxidase localized in the inner mitochondrial membrane of COS-7 cells. The reconstructed dual-color photoswitching image (expanded section) is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed successively in PBS, pH 7.4 in the presence of 100 mM mercapto ethylamine at laser powers of 30 mW at 647 nm and 514 nm with a frame rate of 20 Hz. 16000 images were taken from the two spectrally different fluorophores (scale bar 5 µm).



Photoswitching microscopy performed with **ATTO 655**. The left side shows a conventional immuno-fluorescence image of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** labeled F(ab')$_2$ fragments. The right side shows photoswitching microscopy image with subdiffraction resolution (scale bar 5 µm). Measurements were performed in PBS, pH 7.4 in the presence of 100 mM mercapto ethylamine at a laser power of 30 mW at 647 nm with a frame rate of 10 Hz.

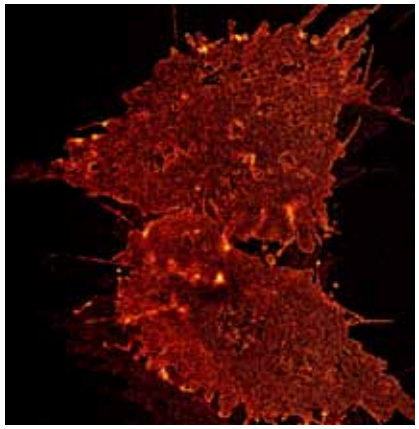
M. Sauer et al., University of Würzburg.



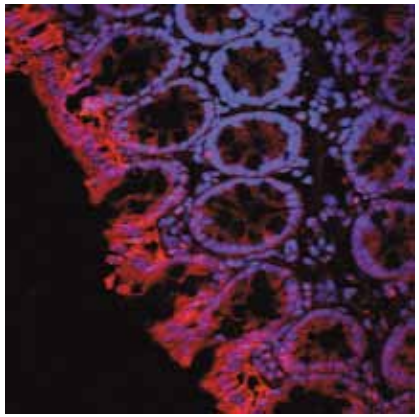
Photoswitching microscopy performed with the two standard fluorophores **ATTO 655** and **ATTO 520**. The upper right part of image A and lower part of image B are conventional immuno-fluorescence images of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** (A) and **ATTO 520** (B) labeled F(ab')$_2$ fragments. Photoswitching microscopy images with subdiffraction resolution (scale bar 1 µm) are superimposed in the lower left (A) and upper (B) part of the images to visualize resolution improvement. Measurements were performed in PBS, pH 7.4 in the presence of 100 mM mercapto ethylamine at a laser power of 30 mW at 647 nm (A) and 514 nm (B) with a frame rate of 10 Hz. (C–F) Evolution of a super-resolution image with **ATTO 520** as fluorophore (1000-16000 frames corresponding to measurement times of 100-1600 s).

M. Sauer et al., University of Würzburg.

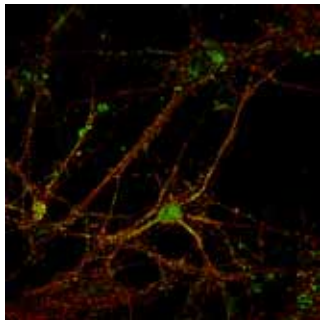
dSTORM super-resolution imaging of the cytoskeletal network of mammalian cells with different ATTO dyes spanning the visible wavelength range (from left to right: **ATTO 520, ATTO 565, ATTO 590, ATTO 655**, and **ATTO 700**). Immuno-fluorescence images (TIRF microscopy) of microtubules in COS-7 cells (10 µm x 10 µm) and corresponding reconstructed dSTORM images are superimposed to highlight the resolution improvement. Experiments were performed in PBS, pH 7.4, 10–200 mM mercapto ethylamine, with a frame rate of 10–20 Hz and excitation intensities of 1-4 kW/cm^2. M. Heilemann et al. Angew. Chem. Int. Ed. 48 (2009) 6903-6908.



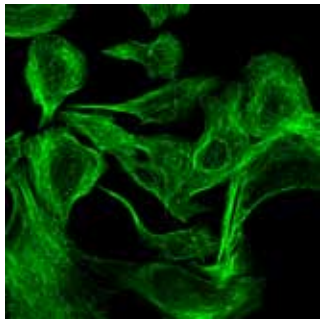
dSTORM image of SLAH3 anion channel in HEK cells labeled with **ATTO 655**.



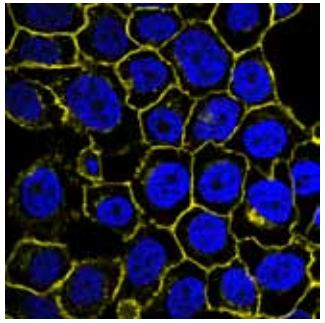
Expression of Aquaporin 3 in rat colon.
Rat colon sections (paraffin-embedded) were stained with Anti-Aquaporin 3-**ATTO 594** antibody (1:100).
Staining (red color) is present in absorptive cells of the colonic epithelium. Hoechst 33342 (blue) is used as counterstain.



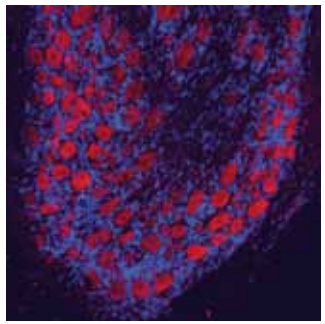
SIM image of hippocampal mouse neuron.
NR1 subunit of the NMDA receptor labeled with **ATTO 532** and Homer labeled with **ATTO 647N**.



Methanol fixed MDA-MB-468 cells; tubulin-antibody staining with mouse IgG anti beta tubulin (primary) and donkey IgG-fab2-fragment anti mouse labeled with **ATTO 488** (secondary).



PFA fixed MDA-MB-468 cells; EGF-receptor stained with **ATTO 565** labeled affibody (yellow), DNA-counterstaining with DAPI (blue).



Expression of NMDAR2B (NR2B) in rat DRG.
Immunohistochemical staining of rat dorsal root ganglia (DRG) frozen sections using Anti-NMDA Receptor 2B (NR2B) (extracellular)-**ATTO 594** antibody (1:50). Staining (red) is present in neuronal cell bodies. Hoechst 33342 is used as the counterstain (blue).

List of Abbreviations

Abbreviation	
λ	wavelength
λ_{abs}	longest-wavelength absorption maximum
ε_{max}	molar decadic extinction coefficient at the longest-wavelength absorption maximum
ε_{260}	molar decadic extinction coefficient at λ = 260 nm
ε_{280}	molar decadic extinction coefficient at λ = 280 nm
CF_{260}	$CF_{260} = \varepsilon_{260} / \varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-DNA conjugates.
CF_{280}	$CF_{280} = \varepsilon_{280} / \varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-protein conjugates.
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	real fluorescence decay time, $\tau_{fl} = \eta_{fl} \times \tau_0$
τ_0	natural (radiative) decay time
η_T	triplet quantum yield
MW	molecular weight
M^+	molecular weight of dye cation (HPLC-MS)
MH^+	molecular weight of protonated dye (HPLC-MS)
An^-	counterion(s)
Δm	increase of molecular mass on conjugation with ATTO-labels
Δq	change of electrical charge on conjugation with ATTO-labels
PBS	phosphate-buffered saline
DOL	degree of labeling
AEDP	(3-[(2-aminoethyl)dithio]propionic acid)
DSPE	1,2 distereoyl-*sn*-glycero-3-phosphoethanolamine
DLPE	1,2 dilauroyl-*sn*-glycero-3-phosphoethanolamine
PEG	linker chain of polyethyleneglycol
DBCO	dibenzocyclooctyne
HUVEC	human umbilical vein endothelial cells
DAPI	4‘,6-diamidino-2-phenylindole
FITC	fluorescein isothiocyanate
TAMRA	6-carboxytetramethylrhodamine
FAM	6-carboxyfluorescein
TET	tetrachloro-6-carboxyfluorescein
JOE	2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein
HEX	hexachloro-6-carboxyfluorescein
ROX	6-carboxy-X-rhodamine
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine

Acknowledgments

ATTO-TEC GmbH is deeply grateful to the following individuals and companies for their contribution to this catalogue by providing fascinating images:

- Prof. Dr. Markus Sauer, Biotechnology and Biophysics, University of Würzburg, Germany
- Prof. Dr. Claus Seidel, Molecular Physical Chemistry, Heinrich Heine University Düsseldorf
- Prof. Dr. Christian Eggeling, Redcliff Department of Medicine, University of Oxford
- Alomone Labs Ltd., Jerusalem, Israel
- Active Motif Corporation, Carlsbad, CA 92008, USA

Catalogue 2016/2018

Headquarters of **ATTO-TEC** *GmbH*