

*Fluorescent Labels
and Dyes*

catalogue 2009/2010

Cover:

Crystals of different fluorescent ATTO dyes gradually dissolving in ethanol.
Photograph: A. Zilles

Inset: Cells labeled with various ATTO dyes (see p. 85).

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ATTO-TEC GmbH Fluorescence - Our Passion

Although the phenomenon as such has been known for more than a century, it was only during the last few decades that *fluorescence* has developed into a powerful tool in biochemistry and medical diagnostics. Applications now have become so diversified and sophisticated that there is an ever growing demand for new and better *fluorescent dyes*.

To take up the challenge **ATTO-TEC** GmbH was founded in 1999. The company has grown continually since. It is staffed by internationally renowned scientists with long-time expertise in dye chemistry and physics. Consequently, ATTO-dyes nowadays are used with great success by scientists throughout the world. Researchers prefer ATTO-products for their high purity and excellent performance. In many applications ATTO-dyes are not merely an alternative, they are the *better* choice.

We are proud to present to you the new edition of our catalogue. In this booklet you will find many new and innovative fluorescent labels - proprietary compounds covered by **ATTO-TEC** patents and patent applications. – Our continuous research is aimed at optimum dye solutions for our customers.

It is a pleasure to introduce you to **ATTO-TEC** – the company that creates success with fluorescent dyes.

The Team of ATTO-TEC



Headquarters of **ATTO-TEC** GmbH

Contact Information

Addresses:

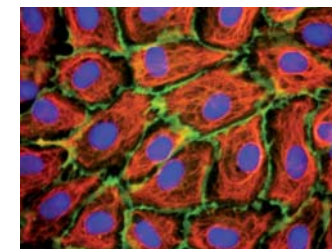
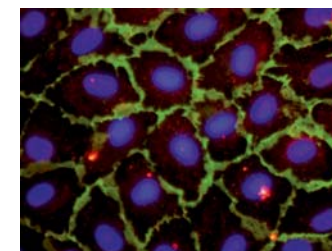
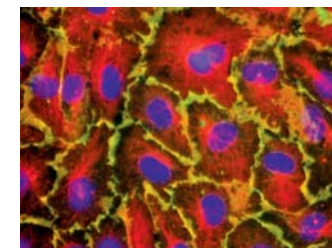
ATTO-TEC GmbH
Am Eichenhang 50
D-57076 Siegen
Germany

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 years. However, it was only during the last few decades that versatile light sources (lasers etc.) and highly sensitive detectors have been developed.

In recent years fluorescence spectroscopy has become a powerful tool with outstanding sensitivity. By sophisticated techniques nowadays even single molecules can be studied via fluorescence. 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 advisable. Secondly the label should show strong absorption at the excitation wavelength as well as high fluorescence quantum yield. 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 the excitation source and a filter set with high transmittance between 650 nm and 750 nm, ATTO 647N would be a very good choice. As can be seen from the list of ATTO-labels in this catalogue, ATTO 647N (p. 50) has a high extinction coefficient at 635 nm (as follows from $\epsilon_{\max}$ and inspection of the absorption curve) as well as an excellent quantum yield of fluorescence ($\eta_f = 0.65$). It is to be noted, however, that besides optical considerations other factors may be important for the choice of label, e.g. pH-dependence, solubility, photostability, size of chromophore or linker and many others.

If there is no label available with an absorption maximum exactly matching the wavelength of the excitation source, a label with a slightly longer wavelength should be chosen. The absorbance will be smaller, but the larger difference

between excitation wavelength and fluorescence (the latter being independent of excitation wavelength with all dyes) has the advantage of better discrimination against scattered excitation light.

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

Light source	Emission line	Best suited dyes
Mercury arc lamp	365 nm	ATTO 390
	405 nm	ATTO 425
	436 nm	ATTO 425, ATTO 465
	546 nm	ATTO 550, ATTO 565
	577 nm	ATTO 590, ATTO Rho101, ATTO 594, ATTO Rho13, ATTO 610, ATTO 611X
Argon-Ion laser	488 nm 514 nm	ATTO 488, ATTO 520, ATTO 520 ATTO 532, ATTO 550
Nd:YAG laser	532 nm	ATTO 532, ATTO Rho6G, ATTO 550, ATTO 565, ATTO Rho11, ATTO Rho12
He-Ne laser	633 nm	ATTO Rho14, ATTO 633, ATTO 647, ATTO 647N
Krypton-Ion laser	647 nm	ATTO 647, ATTO 647N, ATTO 655, ATTO Oxa12, ATTO 665, ATTO 680
	676 nm	ATTO 665, ATTO 680, ATTO 700, ATTO 725, ATTO 740
Diode laser	635 nm	ATTO 633, ATTO 647, ATTO 647N, ATTO 655

Fluorescence Resonance Energy Transfer (FRET)

FRET is becoming more and more 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 to the other. The rate of energy transfer k_{ET} is in good approximation given by (Förster theory):

$$k_{ET} = \frac{9 \ln 10}{128 \pi^5} \cdot \frac{\kappa^2}{N_A n^4 \tau_0 r^6} \int_0^\infty F(\lambda) \cdot \varepsilon(\lambda) \cdot \lambda^4 d\lambda$$

N_A	Avogadro constant
n	index of refraction
τ_0	radiative decay time of donor
r	distance between donor and acceptor molecule
$F(\lambda)$	fluorescence spectrum of donor, normalized according $\int F(\lambda) d\lambda = 1$
$\varepsilon(\lambda)$	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. Thus FRET is very efficient only when donor and acceptor are in close proximity. With typical dye molecules it becomes negligibly small at distances above 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:

$$R_0^6 = \frac{9 \ln 10}{128 \pi^5} \cdot \frac{\kappa^2 \eta_{fl}}{N_A n^4} \int_0^\infty F(\lambda) \cdot \varepsilon(\lambda) \cdot \lambda^4 d\lambda$$

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. 12-13. These values have been calculated with the assumption of statistical orientation of both donor and acceptor (orientation factor $\kappa^2 = 2/3$), a situation typically encountered in solutions of unbound dye molecules. However, in case of dye labeled biomolecules the chromophores of donor and acceptor may be held rigidly in a fixed position. 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.

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

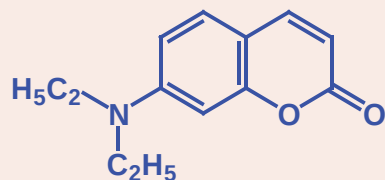
Donor	Acceptor												
ATTO	390	425	465	488	495	520	532	540Q	550	565	580Q	590	594
390	14	41	50	58	59	60	56	54	53	52	47	48	45
425		36	46	59	59	61	58	56	56	55	51	51	49
465			35	52	51	61	56	55	56	55	53	54	52
488				50	46	61	64	63	63	63	60	60	57
495					41	56	56	56	57	58	55	56	54
520						57	65	66	67	67	64	64	61
532							57	63	68	68	67	68	66
550									58	63	69	70	68
565										61	69	72	71
590												63	66
594													62
610													
611X													
620													
633													
647													
647N													
655													
680													
700													
725													
740													
610	610	611X	612Q	620	633	647	647N	655	680	700	725	740	ATTO
44	40	43	41	41	39	39	40	38	35	36	36	36	390
48	44	47	45	45	43	43	43	41	38	36	37	37	425
52	49	52	49	49	48	48	48	46	43	41	40	40	465
57	53	55	53	53	51	51	50	48	44	41	40	40	488
54	51	53	51	51	49	49	49	47	44	42	41	41	495
60	56	59	56	55	54	53	53	50	46	43	41	41	520
66	62	64	61	61	60	59	59	57	53	50	48	48	532
69	65	67	67	66	65	65	64	62	58	55	53	53	550
73	69	70	69	69	69	68	68	65	61	58	56	56	565
73	69	71	71	73	73	74	73	71	69	66	63	63	590
70	67	68	70	73	74	75	75	74	72	69	68	68	594
64	64	63	66	70	72	73	76	75	74	69	68	68	610
	44	44	45	50	57	56	62	65	66	65	64	64	611X
			58	64	68	70	70	69	68	67	65	65	620
				60	68	69	72	73	72	72	71	71	633
					51	52	58	60	61	61	60	60	647
						65	72	75	74	73	72	72	647N
							58	64	66	66	65	65	655
								59	65	67	66	66	680
									58	66	66	700	700
										52	56	725	725
											54	740	740

Molecular Structure of Fluorescent Labels

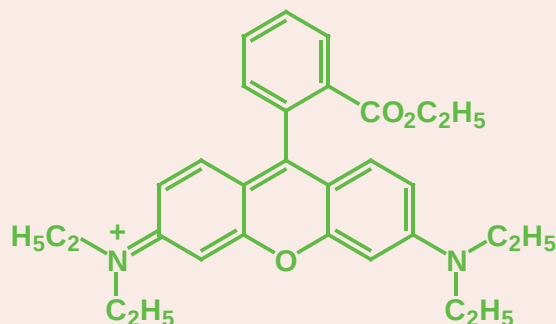
The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of 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:

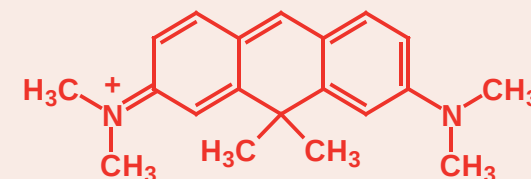
- Coumarin**



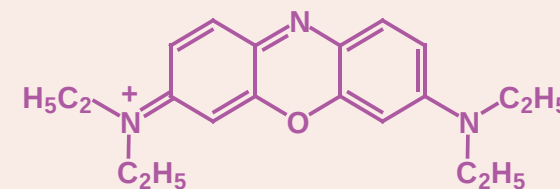
- Rhodamine**



- Carbopyronin**

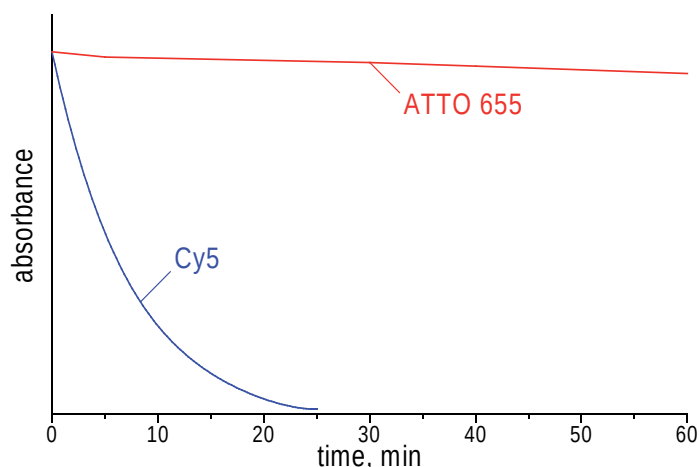


- Oxazine**



Properties of Fluorescent Labels

Apart from absorption and fluorescence there are many other properties that are 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 (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.

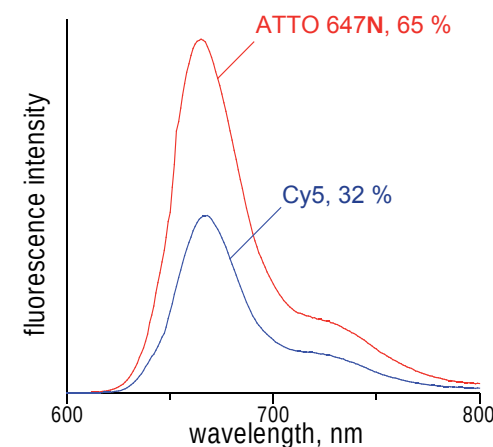


Photostability of ATTO 655 compared with common Cy5TM in water. Irradiation with a 250 W tungsten-halogen lamp. Absorbance vs. time of illumination.

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 new dyes **ATTO 647N** and **ATTO 655** last up to **100 times longer** than dyes like the older Cy5TM and Alexa 647TM. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Besides reduced background, an advantage of excitation in the red spectral region is that rugged diode lasers are readily available. Diode lasers are generally less expensive and more energy-efficient than gas lasers. Furthermore a variety of sensitive detectors is available for the visible-near-IR region. Excitation in the red spectral region is also advantageous when working with live cells, because damage is reduced.

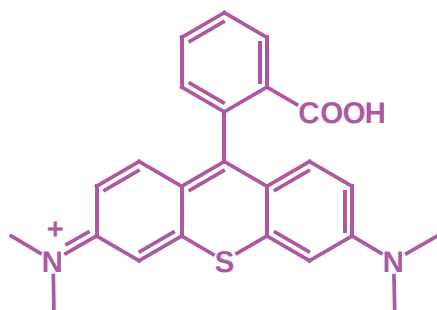
The fluorescence efficiency of dyes is highest in the blue and green region of the spectrum. Here the quantum yield reaches in some cases almost the theoretical limit of 100 %. Towards longer wavelengths the efficiency of dyes drops drastically, in particular so in aqueous solution. However, **ATTO-TEC** has been able to develop labels that show high quantum yield even around 650 nm: The new **ATTO 647N** fluoresces in aqueous solution twice as strong as the older Cy5TM.



Fluorescence quantum yield of **ATTO 647N** compared with common Cy5TM in water. Solutions of equal absorbance excited at 647 nm. Fluorescence intensity vs. wavelength.

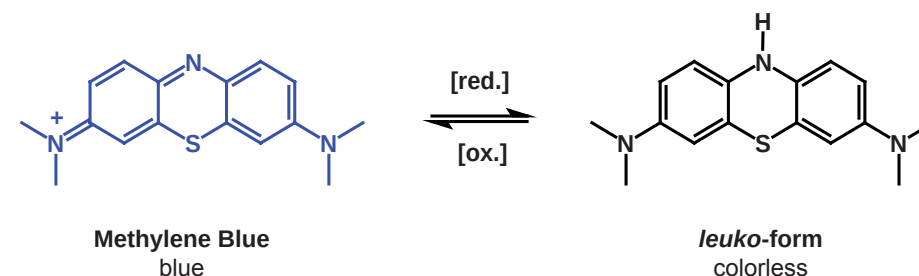
Triplet Labels

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 (singlet oxygen). In addition to the acridine dyes **ATTO 465** (p. 28) and **ATTO 495** (p. 30), both absorbing below 500 nm, we supply **ATTO Thio12** (p. 39), a triplet label derived from *Thiorhodamine*.



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). These interconversions can be catalyzed enzymatically.



Methylene Blue as such cannot be coupled to biomolecules, because it lacks the necessary reactive groups. However, **ATTO-TEC** offers **ATTO MB2** a Methylene Blue derivative featuring a carboxylic acid functionality for coupling (p. 59). The dye is further available as NHS-ester for direct coupling to amino groups or as maleimide derivative for coupling to thiol groups. ATTO-TEC also supplies a biotin conjugate for binding to avidin or streptavidin. Additional conjugates can be prepared on request.

Reactive Labels and Conjugates

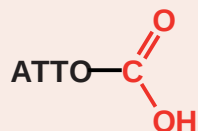
ATTO-labels are designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of most labels are strong absorption, high fluorescence quantum yield, excellent **photostability**, exceptionally high **ozone resistance**, and good water solubility.

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 amine, azide (Click-Chemistry) and iodoacetamide. Dyes with other reactive substituents can be supplied on request.

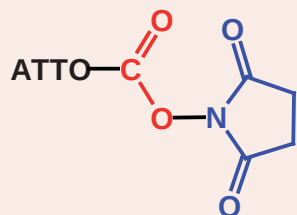
Furthermore a variety of ATTO-dyes are available as phalloidin and streptavidin conjugates. The high affinity of streptavidin to biotin is the basis for the widespread use of streptavidin conjugates. In this connection all ATTO-dyes are also offered as biotin conjugates. ATTO dyes conjugated to other biomolecules like sec. antibodies, lipids, bungarotoxin etc. are available on request.

ATTO Derivatives and Conjugates

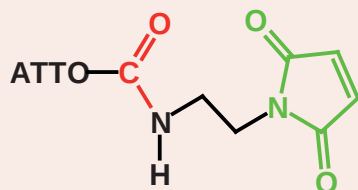
ATTO-dye with free COOH:



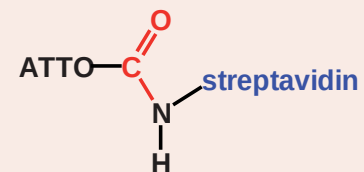
NHS-ester:



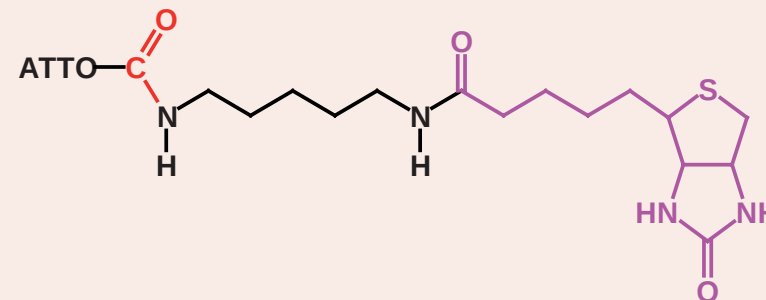
Maleimide:



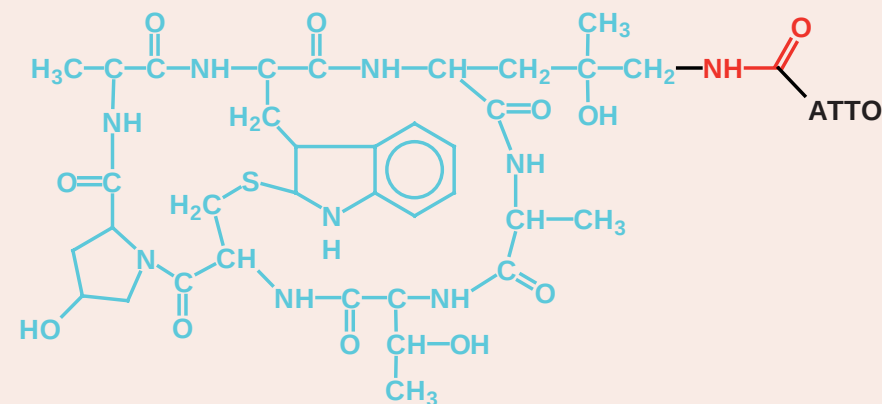
Streptavidin conjugate:



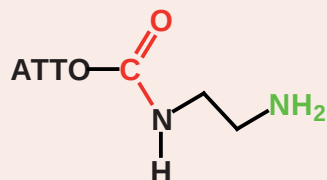
Biotin conjugate:



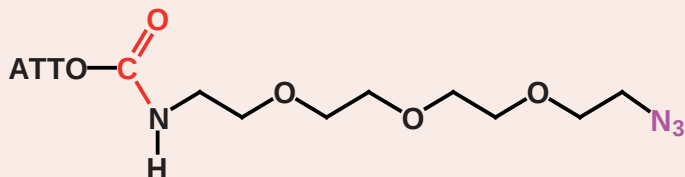
Phalloidin conjugate:



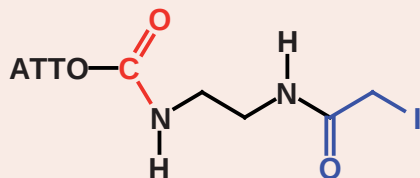
Amine:



Azide:



Iodoacetamide:



About this Catalogue

All spectral data given have been measured on aqueous solutions of the dyes with free carboxy group. When there was a tendency to aggregate, the solution was diluted sufficiently to exhibit the monomeric 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 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 (free COOH, NHS-ester, etc.). In particular the fluorescence quantum yield of the maleimide may be reduced compared to the dye with free 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 (*Products*, or *Support - Downloads - Datable*).
- The correction factors CF_{260} and CF_{280} aide with calculating the degree of labeling (DOL), see „Labeling Procedures“ (p. 68-73).

The molecular weight (*MW*) given has the common meaning, i.e. it refers to the dye including counterions. For mass spectrometry purposes the mass of the dye cation (M^+ or MH^+) is given. Counterions - no matter, what their charge or mass - do not play any role in the labeling reactions with biomolecules.

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

ATTO Fluorescent Labels

Label	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹	λ_{fl} , nm	η_{fl} , %	τ_{fl} , ns	Substitute for
ATTO 390	390	24000	479	90	3.8	
ATTO 425	436	45000	484	90	3.5	
ATTO 465	453	75000	508	55	2.2	
ATTO 488	501	90000	523	80	3.2	Alexa 488*, FITC, FAM**
ATTO 495	495	80000	527	45	2.4	
ATTO 520	516	110000	538	90	3.8	JOE**, TET**
ATTO 532	532	115000	553	90	3.8	Alexa 532*, HEX**
ATTO Rho6G	535	115000	560	90		HEX**
ATTO 550	554	120000	576	80	3.2	TAMRA**, Cy3***
ATTO 565	563	120000	592	90	3.4	Cy3.5***, ROX**
ATTO Rho3B	565	120000	592			
ATTO Rho11	571	120000	595	80		ROX**
ATTO Rho12	576	120000	601	80		
ATTO Thio12	579	110000	609	15		
ATTO Rho101	586	120000	610	80		
ATTO 590	594	120000	624	80	3.7	Alexa 594*, Texas Red*
ATTO Rho13	600	120000	625	80		
ATTO 594	601	120000	627	85	3.5	Alexa 594*
ATTO 610	615	150000	634	70	3.3	
ATTO 611X	611	100000	681	35	2.5	
ATTO 620	619	120000	643	50	2.9	
ATTO Rho14	625	140000	646	80		Alexa 633*
ATTO 633	629	130000	657	64	3.2	Alexa 633*
ATTO 647	645	120000	669	20	2.3	Cy5***, Alexa 647*
ATTO 647N	644	150000	669	65	3.4	Cy5***, Alexa 647*
ATTO 655	663	125000	684	30	1.9	Cy5***, Alexa 647*
ATTO Oxa12	663	125000	684	30		
ATTO 665	663	160000	684	60		
ATTO 680	680	125000	700	30	1.8	Cy5.5***
ATTO 700	700	120000	719	25	1.5	Cy5.5***
ATTO 725	729	120000	752	10	0.5	
ATTO 740	740	120000	764	10	0.6	

ATTO Fluorescence Quenchers

Label	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹	Quenching Range, nm
ATTO 540Q	542	105000	500 - 565
ATTO 580Q	586	110000	535 - 610
ATTO 612Q	615	115000	555 - 640

ATTO-Dyes with Large Stokes-Shift

Label	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹	λ_{fl} , nm	η_{fl} , %
ATTO 390	390	24000	479	90
ATTO 425	436	45000	484	90
ATTO 465	453	75000	508	55
ATTO 611X	611	100000	681	35

ATTO Triplet Label

Label	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹	λ_{fl} , nm	η_{T} , %
ATTO 465	453	75000	508	10
ATTO 495	495	80000	527	10
ATTO Thio12	579	110000	609	20

ATTO Redox Label

Label	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹
ATTO MB2	658	110000

λ_{abs}	longest-wavelength absorption maximum
ϵ_{max}	molar extinction coefficient at the longest-wavelength absorption maximum
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	fluorescence decay time
τ_0	natural (radiative) fluorescence decay time
MW	molecular weight
M ⁺	molecular weight of dye cation (HPLC-MS)
MH ⁺	molecular weight of protonated dye (HPLC-MS)
CF ₂₆₀	CF ₂₆₀ = $\epsilon_{260}/\epsilon_{\text{max}}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-DNA conjugates.
CF ₂₈₀	CF ₂₈₀ = $\epsilon_{280}/\epsilon_{\text{max}}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-protein conjugates.

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

ATTO 390

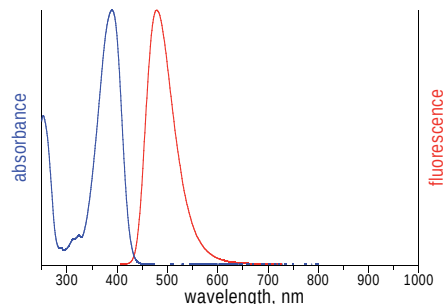
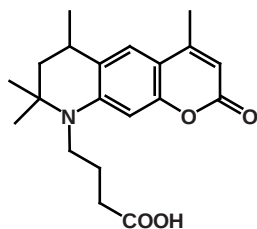


Optical properties of carboxy derivative

λ_{abs}	=	390 nm	
ϵ_{max}	=	$2.4 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	479 nm	
η_{fl}	=	90 %	$\text{CF}_{260} = 0.52$
τ_{fl}	=	3.8 ns	$\text{CF}_{280} = 0.08$

Features:

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



Modification	MW, g/mol	MH ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	343	344	AD 390-21	AD 390-25
NHS-ester	440	441	AD 390-31	AD 390-35
maleimide	465	466	AD 390-41	AD 390-45
biotin	653	654	AD 390-71	AD 390-75
phalloidin	1226	1113	AD 390-81*	AD 390-82**

* 10 nmol **20 nmol

Other conjugates with streptavidin, sec. antibodies etc. on request.

ATTO 425

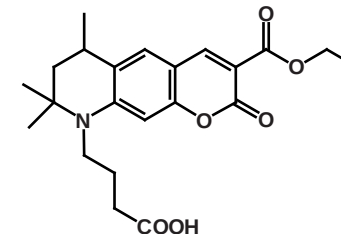
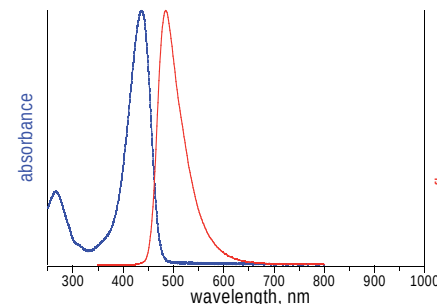


Optical properties of carboxy derivative

λ_{abs}	=	436 nm	
ϵ_{max}	=	$4.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	484 nm	
η_{fl}	=	90 %	$\text{CF}_{260} = 0.27$
τ_{fl}	=	3.5 ns	$\text{CF}_{280} = 0.23$

Features:

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



Modification	MW, g/mol	MH ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	401	402	AD 425-21	AD 425-25
NHS-ester	498	499	AD 425-31	AD 425-35
maleimide	523	524	AD 425-41	AD 425-45
streptavidin			AD 425-61	AD 425-65
biotin	711	712	AD 425-71	AD 425-75
phalloidin	1285	1172	AD 425-81*	AD 425-82**

* 10 nmol **20 nmol

Other conjugates with sec. antibodies etc. on request.

ATTO 465

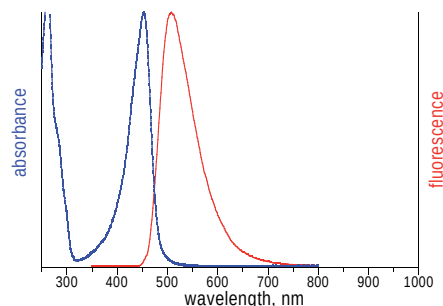
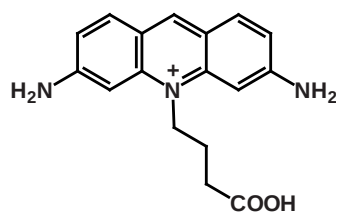


Optical properties of carboxy derivative

λ_{abs}	=	453 nm	
ϵ_{max}	=	$7.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	508 nm	
η_{fl}	=	55 %	$\text{CF}_{260} = 1.12$
τ_{fl}	=	2.2 ns	$\text{CF}_{280} = 0.54$

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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 488

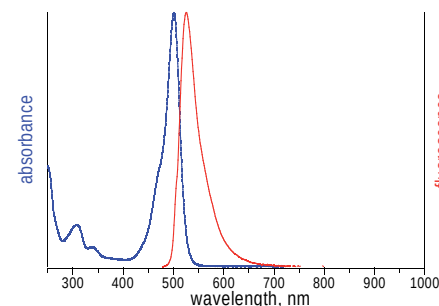


Optical properties of carboxy derivative

λ_{abs}	=	501 nm	
ϵ_{max}	=	$9.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	523 nm	
η_{fl}	=	80 %	$\text{CF}_{260} = 0.25$
τ_{fl}	=	3.2 ns	$\text{CF}_{280} = 0.10$

Features:

- High fluorescence yield
- High photostability
- **Very hydrophilic**
- Excellent water solubility
- **Very little aggregation**
- New dye with net charge of -1



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1472	1359	AD 488-81*	AD 488-82**
amine	858	632	AD 488-91	AD 488-95
azide	903	790	AD 488-101	AD 488-105
iodoacetamide	913	800	AD 488-111	AD 488-115

* 10 nmol **20 nmol

ATTO 495

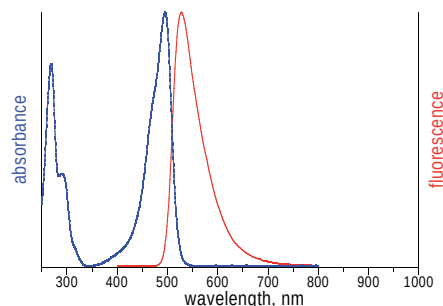
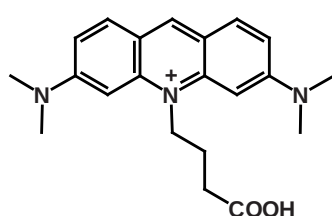


Optical properties of carboxy derivative

λ_{abs}	=	495 nm	
ϵ_{max}	=	$8.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	527 nm	
η_{fl}	=	45 %	$\text{CF}_{260} = 0.57$
τ_{fl}	=	2.4 ns	$\text{CF}_{280} = 0.39$

Features:

- High triplet yield
- Phosphorescent in solid matrix
- Moderately hydrophilic
- Cationic dye derived from well-known Acridine Orange



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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			AD 495-61	AD 495-65
biotin	762	662	AD 495-71	AD 495-75

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 520

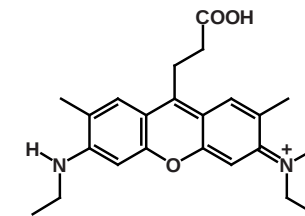
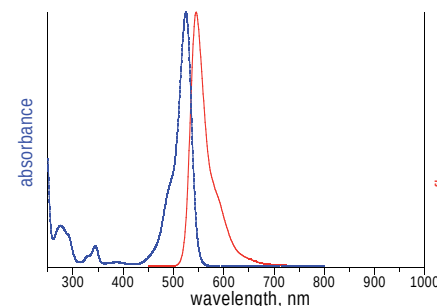


Optical properties of carboxy derivative

λ_{abs}	=	516 nm	
ϵ_{max}	=	$1.1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	538 nm	
η_{fl}	=	90 %	$\text{CF}_{260} = 0.13$
τ_{fl}	=	3.8 ns	$\text{CF}_{280} = 0.18$

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- 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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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

Other conjugates with phalloidin etc. on request.

ATTO 532

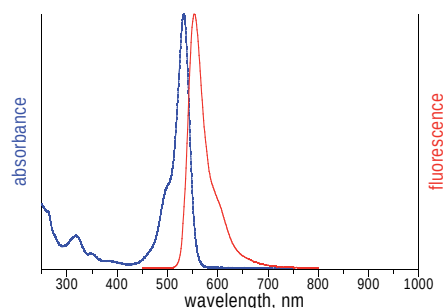
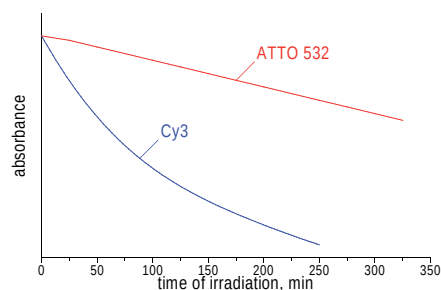


Optical properties of carboxy derivative

λ_{abs}	=	532 nm	
ϵ_{max}	=	$1.15 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	553 nm	
η_{fl}	=	90 %	$\text{CF}_{260} = 0.22$
τ_{fl}	=	3.8 ns	$\text{CF}_{280} = 0.11$

Features:

- High fluorescence yield
- High photostability
- **Very hydrophilic**
- Excellent water solubility
- **Very little aggregation**
- New dye with net charge of -1



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1417	AD 532-81*	AD 532-82**
amine	914	688	AD 532-91	AD 532-95
azide	959	846	AD 532-101	AD 532-105
iodoacetamide	969	856	AD 532-111	AD 532-115

* 10 nmol **20 nmol



ATTO Rho6G

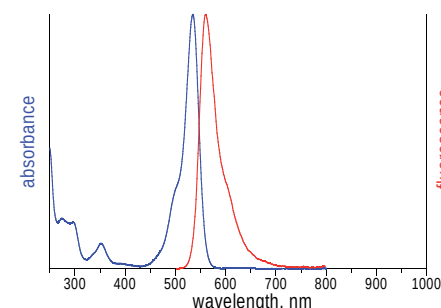


Optical properties of carboxy derivative

λ_{abs}	=	535 nm	
ϵ_{max}	=	$1.15 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	560 nm	$\text{CF}_{260} = 0.22$
η_{fl}	=	90 %	$\text{CF}_{280} = 0.19$

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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	614	514	AD Rho6G-21	AD Rho6G-25
NHS-ester	711	611	AD Rho6G-31	AD Rho6G-35
maleimide	749	636	AD Rho6G-41	AD Rho6G-45
biotin	937	824	AD Rho6G-71	AD Rho6G-75
phalloidin	1396	1283	AD Rho6G-81*	AD Rho6G-82**

* 10 nmol **20 nmol

Other conjugates with streptavidin, sec. antibodies etc. on request.

ATTO 550

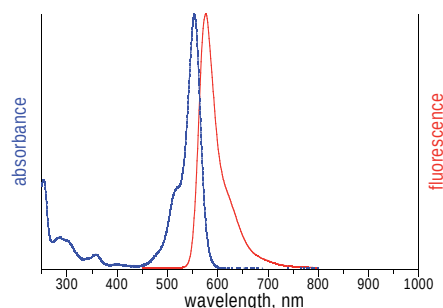


Optical properties of carboxy derivative

λ_{abs}	=	554 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	576 nm	
η_{fl}	=	80 %	$\text{CF}_{260} = 0.24$
τ_{fl}	=	3.2 ns	$\text{CF}_{280} = 0.12$

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye
- Supplied as mixture of three diastereomers



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1004	904	AD 550-71	AD 550-75
phalloidin	1476	1363	AD 550-81*	AD 550-82**
amine	862	636	AD 550-91	AD 550-95
azide	907	794	AD 550-101	AD 550-105
iodoacetamide	917	804	AD 550-111	AD 550-115

* 10 nmol **20 nmol

ATTO 565

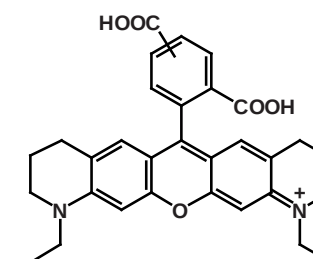
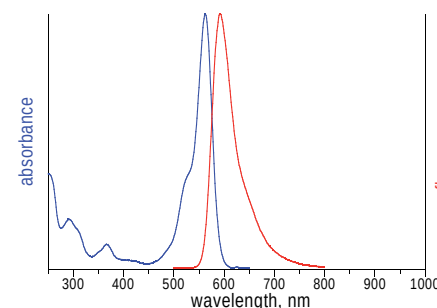


Optical properties of carboxy derivative

λ_{abs}	=	563 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	592 nm	
η_{fl}	=	90 %	$\text{CF}_{260} = 0.34$
τ_{fl}	=	3.4 ns	$\text{CF}_{280} = 0.16$

Features:

- High fluorescence yield
- High thermal and photo-stability
- Cationic dye
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	921	821	AD 565-71	AD 565-75
phalloidin	1393	1280	AD 565-81*	AD 565-82**
amine	666	553	AD 565-91	AD 565-95
azide	824	711	AD 565-101	AD 565-105
iodoacetamide	834	721	AD 565-111	AD 565-115

* 10 nmol **20 nmol

ATTO Rho3B

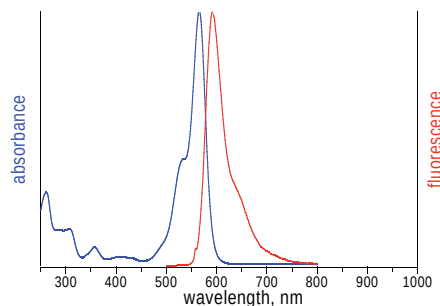
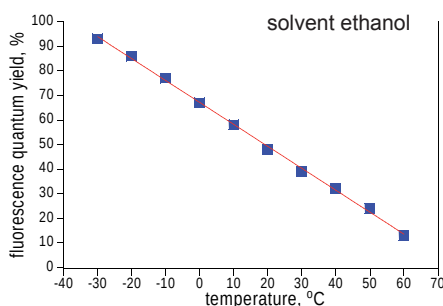


Optical properties of carboxy derivative

λ_{abs}	=	565 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	592 nm	$\text{CF}_{260} = 0.28$
η_{fl}	=	50 % (in ethanol, 20°C)	$\text{CF}_{280} = 0.14$

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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	965	852	AD Rho3B-71	AD Rho3B-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO Rho11

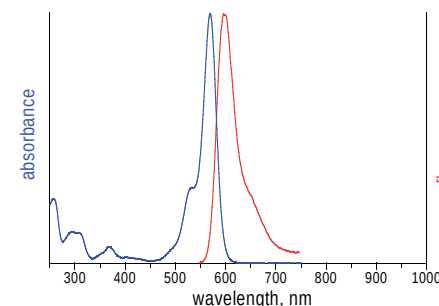


Optical properties of carboxy derivative

λ_{abs}	=	571 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	595 nm	$\text{CF}_{260} = 0.25$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.09$

Features:

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



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	666	566	AD Rho11-21	AD Rho11-25
NHS-ester	763	664	AD Rho11-31	AD Rho11-35
maleimide	788	688	AD Rho11-41	AD Rho11-45
biotin	990	877	AD Rho11-71	AD Rho11-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO Rho12

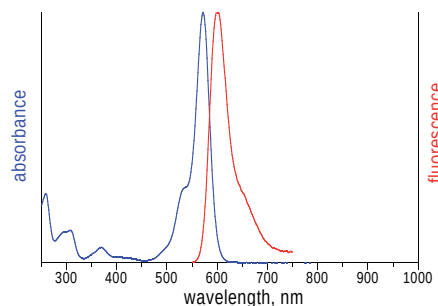


Optical properties of carboxy derivative

λ_{abs}	=	576 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	601 nm	$\text{CF}_{260} = 0.27$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.09$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1073	960	AD Rho12-71	AD Rho12-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO Thio12

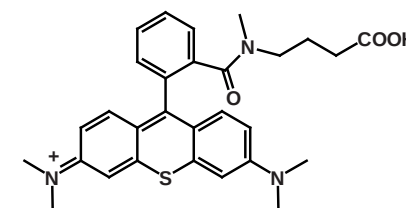
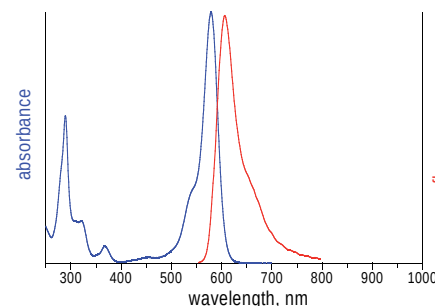


Optical properties of carboxy derivative

λ_{abs}	=	579 nm	
ϵ_{max}	=	$1.1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	609 nm	$\text{CF}_{260} = 0.10$
η_{fl}	=	15 %	$\text{CF}_{280} = 0.37$
η_{T}	=	20 %	

Features:

- High thermal and photo-stability
- High triplet yield
- Moderate fluorescence yield
- Cationic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	602	502	AD Thio12-21	AD Thio12-25
NHS-ester	699	600	AD Thio12-31	AD Thio12-35
maleimide	724	624	AD Thio12-41	AD Thio12-45
biotin	925	812	AD Thio12-71	AD Thio12-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO Rho101

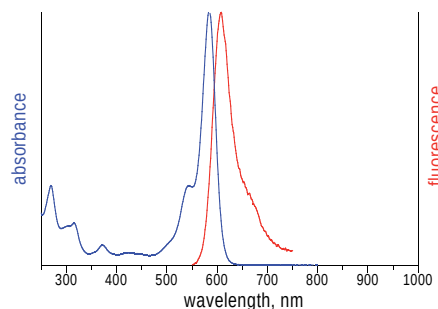


Optical properties of carboxy derivative

λ_{abs}	=	586 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	610 nm	$\text{CF}_{260} = 0.24$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.19$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1013	900	AD Rho101-71	AD Rho101-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 590

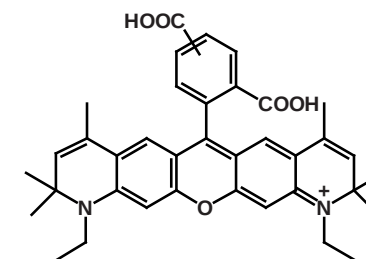
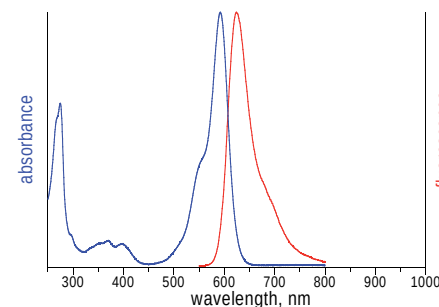


Optical properties of carboxy derivative

λ_{abs}	=	594 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	624 nm	$\text{CF}_{260} = 0.42$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.44$
τ_{fl}	=	3.7 ns	

Features:

- High fluorescence yield
- High thermal and photo-stability
- New dye related to rhodamines
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1001	901	AD 590-71	AD 590-75
phalloidin	1473	1360	AD 590-81*	AD 590-82**
amine	916	689	AD 590-91	AD 590-95
azide	904	791	AD 590-101	AD 590-105
iodoacetamide	970	857	AD 590-111	AD 590-115

* 10 nmol **20 nmol

ATTO 594

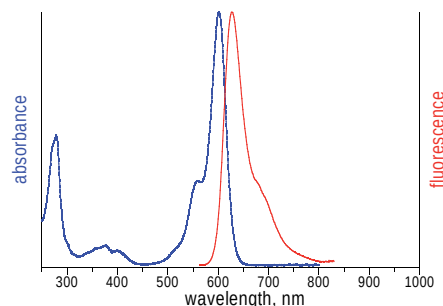


Optical properties of carboxy derivative

λ_{abs}	=	601 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	627 nm	
η_{fl}	=	85 %	$\text{CF}_{260} = 0.26$
τ_{fl}	=	3.5 ns	$\text{CF}_{280} = 0.51$

Features:

- High fluorescence yield
- High photostability
- **Very hydrophilic**
- Excellent water solubility
- **Very little aggregation**
- New dye with net charge of -1



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
amine	1174	948	AD 594-91	AD 594-95

Other conjugates with sec. antibodies, phalloidin etc. on request.



ATTO Rho13

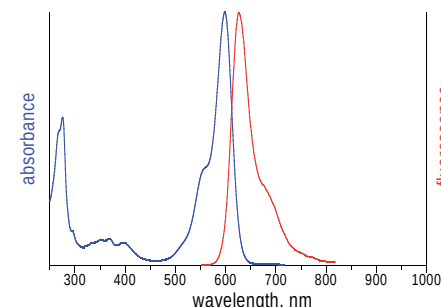


Optical properties of carboxy derivative

λ_{abs}	=	600 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	625 nm	$\text{CF}_{260} = 0.10$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.37$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	745	646	AD Rho13-21	AD Rho13-25
NHS-ester	843	743	AD Rho13-31	AD Rho13-35
maleimide	867	768	AD Rho13-41	AD Rho13-45
biotin	1069	956	AD Rho13-71	AD Rho13-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 610

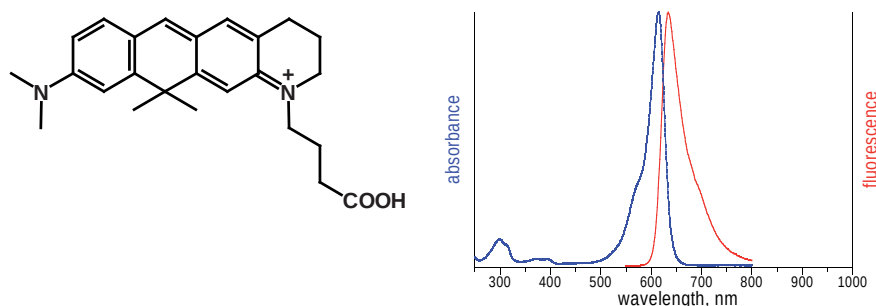


Optical properties of carboxy derivative

λ_{abs}	=	615 nm	
ϵ_{max}	=	$1.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	634 nm	
η_{fl}	=	70 %	$\text{CF}_{260} = 0.02$
τ_{fl}	=	3.3 ns	$\text{CF}_{280} = 0.05$

Features:

- High fluorescence yield
- High photostability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Cationic dye belonging to new class of carbopyronins



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	801	701	AD 610-71	AD 610-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 611X

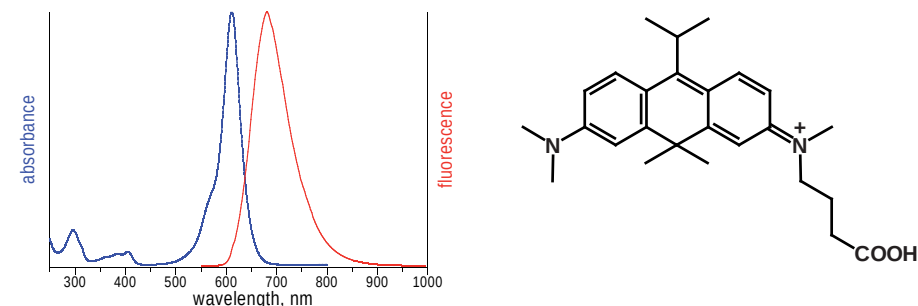


Optical properties of carboxy derivative

λ_{abs}	=	611 nm	
ϵ_{max}	=	$1.0 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	681 nm	
η_{fl}	=	35 %	$\text{CF}_{260} = 0.05$
τ_{fl}	=	2.5 ns	$\text{CF}_{280} = 0.07$

Features:

- Fluorescence yield unusually high in this wavelength region
- **Large Stokes-shift**
- High photostability
- At pH > 5 reversible formation of colorless pseudobase
- Cationic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	494	407	AD 611X-21	AD 611X-25
NHS-ester	591	504	AD 611X-31	AD 611X-35
maleimide	616	529	AD 611X-41	AD 611X-45
biotin	804	717	AD 611X-71	AD 611X-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 620

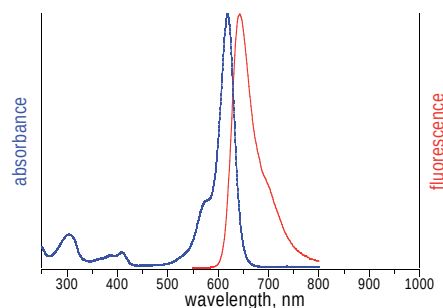


Optical properties of carboxy derivative

λ_{abs}	=	619 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	643 nm	
η_{fl}	=	50 %	$\text{CF}_{260} = 0.05$
τ_{fl}	=	2.9 ns	$\text{CF}_{280} = 0.07$

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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	922	822	AD 620-71	AD 620-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.



ATTO Rho14

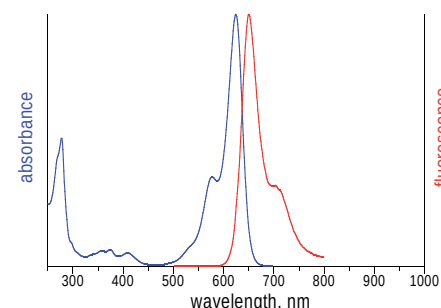


Optical properties of carboxy derivative

λ_{abs}	=	625 nm	
ϵ_{max}	=	$1.4 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	646 nm	$\text{CF}_{260} = 0.29$
η_{fl}	=	80 %	$\text{CF}_{280} = 0.46$

Features:

- Fluorescence yield unusually high in this wavelength region
- High thermal and photo-stability
- Cationic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	897	784	AD Rho14-21	AD Rho14-25
NHS-ester	981	881	AD Rho14-31	AD Rho14-35
maleimide	1019	906	AD Rho14-41	AD Rho14-45
biotin	1221	1094	AD Rho14-71	AD Rho14-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 633

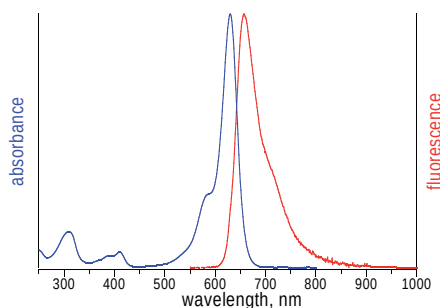
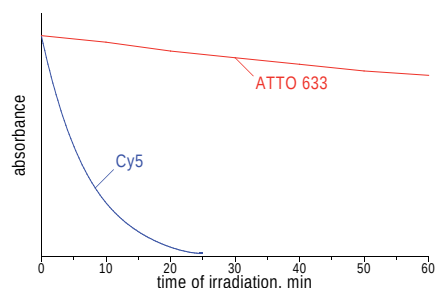


Optical properties of carboxy derivative

λ_{abs}	=	629 nm	
ϵ_{max}	=	$1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	657 nm	
η_{fl}	=	64 %	$\text{CF}_{260} = 0.05$
τ_{fl}	=	3.2 ns	$\text{CF}_{280} = 0.06$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	962	862	AD 633-71	AD 633-75
phalloidin	1434	1321	AD 633-81*	AD 633-82**
amine	707	594	AD 633-91	AD 633-95
azide	865	752	AD 633-101	AD 633-105
iodoacetamide	875	762	AD 633-111	AD 633-115

* 10 nmol **20 nmol

ATTO 647

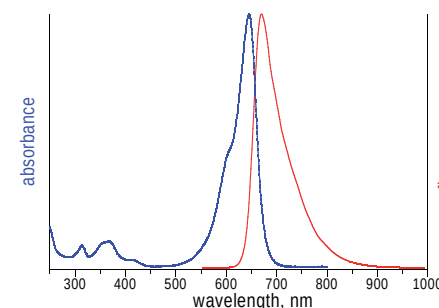


Optical properties of carboxy derivative

λ_{abs}	=	645 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	669 nm	
η_{fl}	=	20 %	$\text{CF}_{260} = 0.08$
τ_{fl}	=	2.3 ns	$\text{CF}_{280} = 0.04$

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Stable at pH 2 - 8
- Zwitterionic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	592	593	AD 647-21	AD 647-25
NHS-ester	811	690	AD 647-31	AD 647-35
maleimide	832	715	AD 647-41	AD 647-45
streptavidin			AD 647-61	AD 647-65
biotin	1219	903	AD 647-71	AD 647-75

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 647N

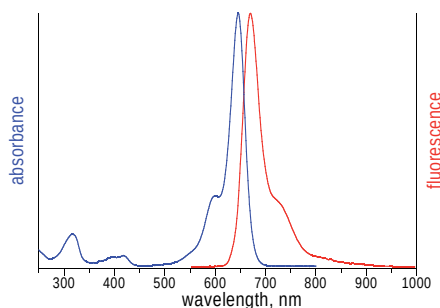
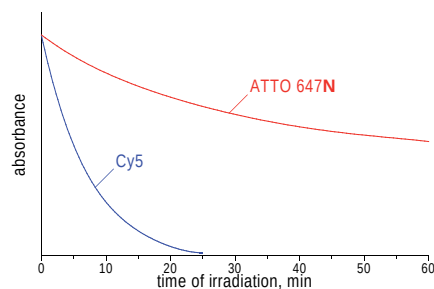


Optical properties of carboxy derivative

λ_{abs}	=	644 nm	
ϵ_{max}	=	$1.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	669 nm	
η_{fl}	=	65 %	$\text{CF}_{260} = 0.06$
τ_{fl}	=	3.4 ns	$\text{CF}_{280} = 0.05$

Features:

- Extraordinarily high fluorescence yield at this wavelength
- High thermal and photo-stability
- Excellent ozone resistance
- Moderately hydrophilic
- Cationic dye, mixture of two isomers



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	746	646	AD 647N-21	AD 647N-25
NHS-ester	843	743	AD 647N-31	AD 647N-35
maleimide	868	768	AD 647N-41	AD 647N-45
streptavidin			AD 647N-61	AD 647N-65
biotin	1056	956	AD 647N-71	AD 647N-75
phalloidin	1528	1415	AD 647N-81*	AD 647N-82**
amine	801	688	AD 647N-91	AD 647N-95
azide	959	846	AD 647N-101	AD 647N-105
iodoacetamide	969	856	AD 647N-111	AD 647N-115

* 10 nmol **20 nmol

ATTO 655

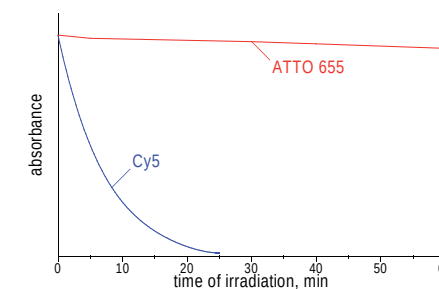
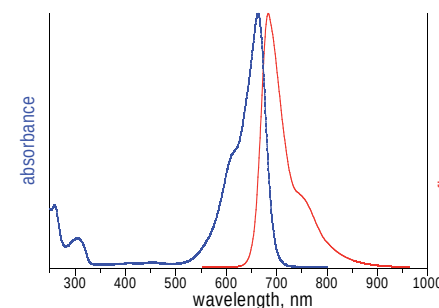


Optical properties of carboxy derivative

λ_{abs}	=	663 nm	
ϵ_{max}	=	$1.25 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	684 nm	
η_{fl}	=	30 %	$\text{CF}_{260} = 0.24$
τ_{fl}	=	1.9 ns	$\text{CF}_{280} = 0.08$

Features:

- High fluorescence yield
- Excellent thermal and photo-stability
- Excellent ozone resistance
- Fluorescence quenching by guanine, tryptophan, etc.
- Very hydrophilic



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1410	1297	AD 655-81*	AD 655-82**
amine	796	570	AD 655-91	AD 655-95
azide	841	728	AD 655-101	AD 655-105
iodoacetamide	851	738	AD 655-111	AD 655-115

* 10 nmol **20 nmol

ATTO Oxa12

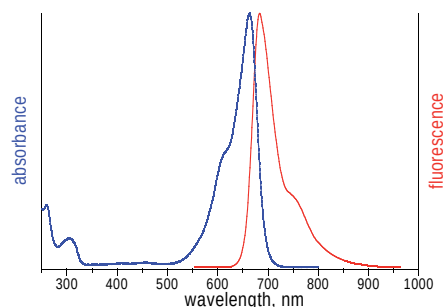


Optical properties of carboxy derivative

λ_{abs}	=	663 nm	
ϵ_{max}	=	$1.25 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	684 nm	$\text{CF}_{260} = 0.24$
η_{fl}	=	30 %	$\text{CF}_{280} = 0.08$

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	
			Unit (1 mg)	Unit (5 mg)
with free COOH	738	639	AD Oxa12-21	AD Oxa12-25
NHS-ester	835	736	AD Oxa12-31	AD Oxa12-35
maleimide	874	761	AD Oxa12-41	AD Oxa12-45
biotin	1062	949	AD Oxa12-71	AD Oxa12-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 665

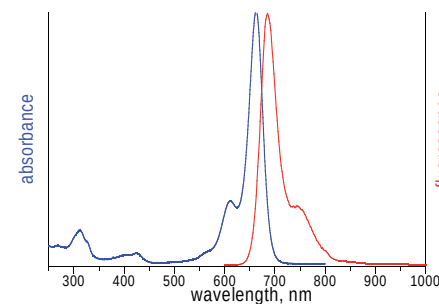


Optical properties of carboxy derivative

λ_{abs}	=	663 nm	
ϵ_{max}	=	$1.60 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	684 nm	$\text{CF}_{260} = 0.07$
η_{fl}	=	60 %	$\text{CF}_{280} = 0.06$

Features:

- **Extraordinarily high fluorescence yield**
- **Excellent thermal and photo-stability**
- **Excellent ozone resistance**
- Moderately hydrophilic



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	1046	933	AD 665-71	AD 665-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 680

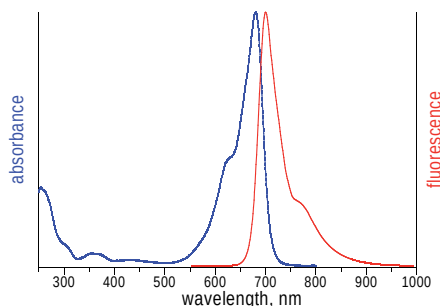


Optical properties of carboxy derivative

λ_{abs}	=	680 nm	
ϵ_{max}	=	$1.25 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	700 nm	
η_{fl}	=	30 %	$CF_{260} = 0.30$
τ_{fl}	=	1.8 ns	$CF_{280} = 0.17$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 700

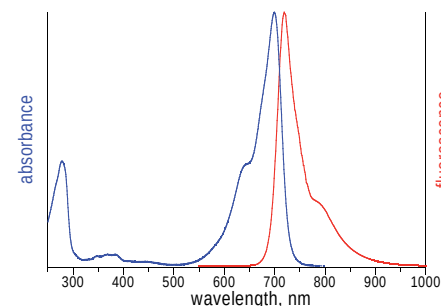


Optical properties of carboxy derivative

λ_{abs}	=	700 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	719 nm	
η_{fl}	=	25 %	$CF_{260} = 0.26$
τ_{fl}	=	1.5 ns	$CF_{280} = 0.41$

Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
amine	722	608	AD 700-91	AD 700-95

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 725

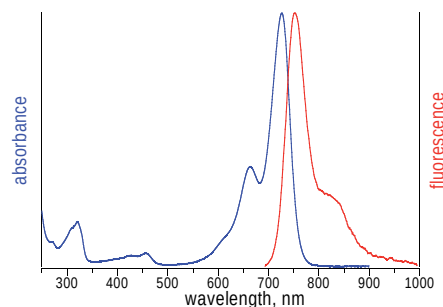
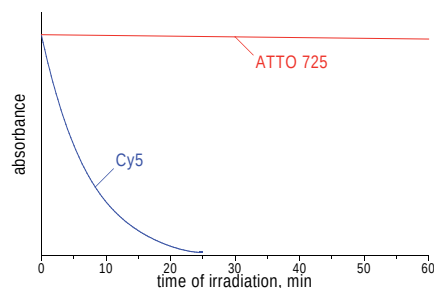


Optical properties of carboxy derivative

λ_{abs}	=	729 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	752 nm	
η_{fl}	=	10 %	$\text{CF}_{260} = 0.10$
τ_{fl}	=	0.5 ns	$\text{CF}_{280} = 0.08$

Features:

- Excellent photostability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Cationic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	826	726	AD 725-71	AD 725-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 740

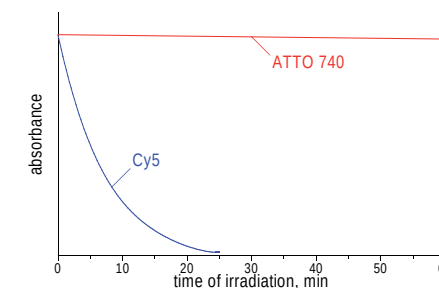
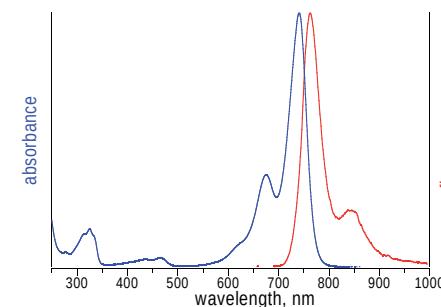


Optical properties of carboxy derivative

λ_{abs}	=	740 nm	
ϵ_{max}	=	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$	
λ_{fl}	=	764 nm	
η_{fl}	=	10 %	$\text{CF}_{260} = 0.11$
τ_{fl}	=	0.6 ns	$\text{CF}_{280} = 0.10$

Features:

- Excellent photostability
- Moderately hydrophilic
- Stable at pH 2 - 8
- Cationic dye



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	878	778	AD 740-71	AD 740-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

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 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.

Optical properties of carboxy derivative

$$\lambda_{\text{abs}} = 658 \text{ nm}$$

$$\varepsilon_{\text{max}} = 1.00 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

$$\text{CF}_{260} = 0.11$$

$$\text{CF}_{280} = 0.28$$

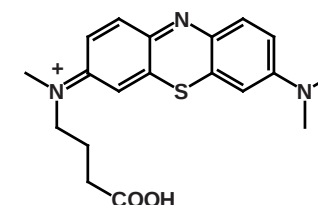
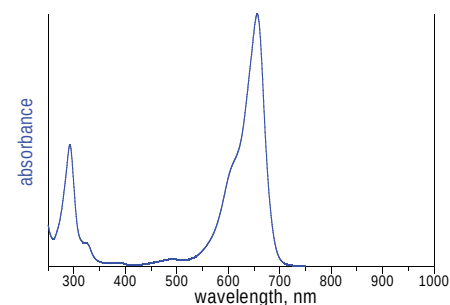


ATTO MB2



Features:

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



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	779	666	AD MB2-71	AD MB2-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

Fluorescence 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. 10-11. 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. 10-11. 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. 61-63. The Förster-radii R_0 for combinations with fluorescent ATTO-labels as donors are presented in the table on p. 12-13.

Note:

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.

ATTO 540Q

Optical properties of carboxy derivative

$$\lambda_{\text{abs}} = 542 \text{ nm}$$

$$\varepsilon_{\text{max}} = 1.05 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

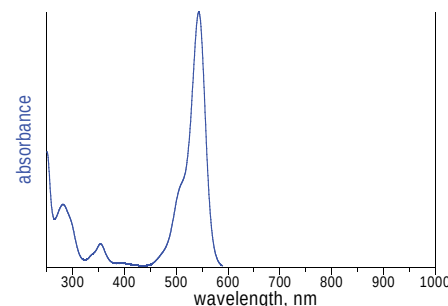
$$\text{CF}_{260} = 0.22$$

$$\text{CF}_{280} = 0.24$$



Features:

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



Modification	MW, g/mol	M^+ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	969	869	AD 540Q-71	AD 540Q-75

Other conjugates with sec. antibodies, phalloidin etc. on request.

ATTO 580Q



Optical properties of carboxy derivative

$$\lambda_{\text{abs}} = 586 \text{ nm}$$

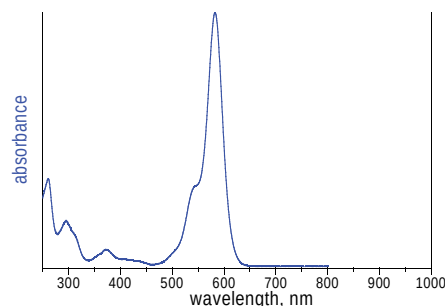
$$\epsilon_{\text{max}} = 1.1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

$$\text{CF}_{260} = 0.36$$

$$\text{CF}_{280} = 0.13$$

Features:

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



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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
biotin	1105	1005	AD 580Q-71	AD 580Q-75

Other conjugates with streptavidin, sec. antibodies, phalloidin etc. on request.

ATTO 612Q



Optical properties of carboxy derivative

$$\lambda_{\text{abs}} = 615 \text{ nm}$$

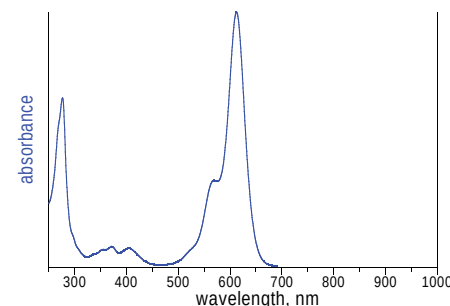
$$\epsilon_{\text{max}} = 1.15 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

$$\text{CF}_{260} = 0.35$$

$$\text{CF}_{280} = 0.57$$

Features:

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



Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
with free COOH	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	1101	1001	AD 612Q-71	AD 612Q-75

Other conjugates with sec. antibodies, phalloidin etc. on request.

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.

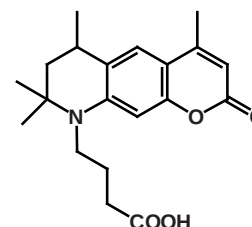
On excitation of dyes with highly *unsymmetrical* π -electron systems the dipole moment may change drastically. The ensuing strong reorientation of solvent molecules leads to an unusually large Stokes-shift, in particular in polar solvents like water and ethanol. As the non-radiative decay of the excited 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 (table p. 24).

Even more remarkable are the dyes **ATTO 465** and **ATTO 611X**. In spite of their symmetrical structure they have large Stokes-shifts of 55 and 70 nm, respectively. The fluorescence quantum yield of 35 % in aqueous solution, measured for **ATTO 611X**, is among the highest found for dyes emitting in the far red region.

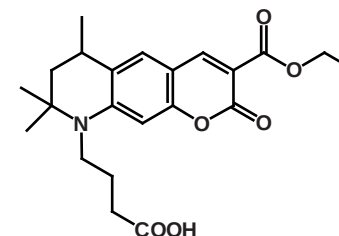
Optical Properties in Water

Label	$\epsilon_{\max}$, M ⁻¹ cm ⁻¹	λ_{abs} , nm	λ_{fl} , nm	Stokes-Shift, nm	η_{fl} , %	τ_{fl} , ns	Page
ATTO 390	24000	390	479	89	90	3.8	26
ATTO 425	45000	436	484	48	90	3.5	27
ATTO 465	75000	453	508	55	55	2.2	28
ATTO 611X	100000	611	681	70	35	2.5	45

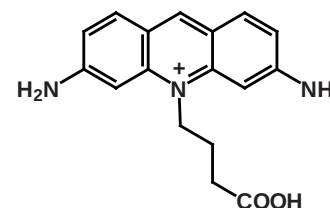
ATTO 390



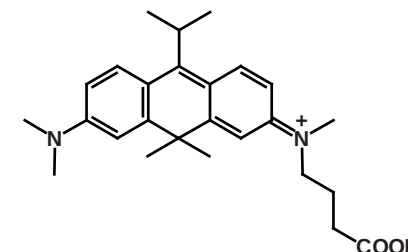
ATTO 425



ATTO 465



ATTO 611X



Customized Labels and Products

In addition to the products described in this catalogue **ATTO-TEC** is pleased to offer on request dyes and labels tailored 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 many applications this has proven to be very suitable and practical. However, if your experiment requires a linker of different length, rigidity, or other special feature, - most likely we are able to provide it.

Reactive Group

N-hydroxysuccinimide (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 label carries an entirely different reactive group: ATTO-TEC can provide amino-, hydrazine-groups, phosphoramidites, products for "Click-Chemistry" (azides, alkynes) and many others.

Solubility, Charges

On customer request ATTO-dyes can be rendered very hydrophobic or else very hydrophilic and thus become compatible with the corresponding solvents, surfaces, or biochemical environments. Cell permeability can be influenced in broad limits. Also dyes may be shielded by a dendrimeric shell. The electrical charge can be adapted to achieve the desired interaction with a biomolecule or simply to obtain a special migration behaviour in electrophoresis.

Conjugates

Fluorescent conjugates of ATTO-dyes with avidin, streptavidin, phalloidin, bungarotoxin, sec. antibodies and lipids such as sphingomyelin, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine etc. are prepared on request.

Special Dyes

Bichromophoric Dyes

If two fluorescent chromophores are connected by a linker, energy transfer (FRET) may occur *intramolecularly*. Thereby the fluorescence of the short-wavelength chromophore is quenched, and fluorescence from the long-wavelength chromophore is observed exclusively. The absorption spectrum of such bichromophoric dye resembles the superposition of the individual spectra. Therefore the dye shows a very strong absorption in a wavelength range considerably wider than in case of a single chromophore, its fluorescence can be excited more efficiently with a broad-band light source. Although bichromophoric dyes are by necessity of larger size than normal labels, they may have an advantage in certain applications. **ATTO-TEC** will supply such dyes on request.

pH-Sensitive Dyes

ATTO-TEC has the capacity to supply various dyes, whose fluorescence efficiency depends strongly on the acidity of the solution - or environment, generally speaking. Depending on the particular molecular structure, such dye will fluoresce in acidic (low pH) or in basic (high pH) environment. The absorption spectrum also may change with pH. Customers are welcome to ask for details.

Protease-Active Dyes

ATTO-TEC has developed a series of dye derivatives which become fluorescent only when activated by the corresponding enzyme (protease). These compounds, very useful for the determination of protease activity, are supplied on request.

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 reagents are N-hydroxysuccinimide(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. Isothiocyanates also react with amino groups. However, in general the resulting thiourea compound is less stable and deteriorates over time. Sulfonyl chlorides are another group of amine-reactive compounds forming very stable sulfonamides, yet are more difficult to work with. Therefore NHS-esters are the preferred amine-reactive reagents for protein- or oligo-conjugation. For the labeling of thiol groups the most popular and commonly used reactive reagents are maleimides. ATTO maleimides react with thiol groups of proteins to form a stable thio-ether bond. Unlike the labeling of amino groups, thiol modifications generally take place at near neutral pH. Since most amino groups show very little reactivity at pH 7, thiol groups can be selectively labeled in the presence of amines.

Labeling Proteins with Amine-Reactive ATTO-Labels (NHS-Esters)

We recommend using 0.1 - 0.2 M sodium bicarbonate buffer of pH 8.3 for labeling proteins. Number and surface position of amino groups vary considerably among different proteins. Therefore it is advisable that different degrees of labeling (DOL) be tried in order to find the most satisfactory solution for the problem at hand.

Procedure

Dissolve 2 - 10 mg of protein in 1 ml of sodium bicarbonate buffer. Protein or peptide solutions must be free of any amine-containing substances such as Tris, free amino acids or ammonium ions. Antibodies that have been previously dissolved in buffers containing amines can be dialyzed against 10 - 20 mM phosphate-buffered saline (PBS), and the desired pH 8.3 for the labeling reaction can be obtained by adding 0.1 ml of 1 M sodium bicarbonate solution for each ml of antibody solution. The presence of low concentrations of sodium azide (< 3 mM) will not interfere with the labeling reaction.

Dissolve 1.0 mg of amine-reactive dye in 100 to 500 μ l of *anhydrous, amine-free* DMF or DMSO. Due to the high quality of ATTO NHS-esters such solutions are stable for a long period of time. However, it may be difficult to avoid humidity entering a solution in continuous use. Hence it is advisable to prepare, whenever possible, the dye solution immediately before starting the labeling reaction. To obtain a degree of labeling (DOL, dye-to-protein ratio) of 2 slowly add, while stirring, a threefold molar excess of reactive dye 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. Incubate the reaction mixture for 1 hour at room temperature. However, in most cases the labeling reaction will be completed within 5-10 minutes. To increase the degree of labeling a higher ratio of NHS-ester to protein has to be used.

Separation of the Conjugate from Free Dye

Part of the applied dye NHS-ester will hydrolyze during the labeling reaction and must be removed from the labeled protein. We recommend using a Sephadex G-25 or equivalent gel filtration column (minimum of 1 cm diameter and 12 cm length; for very hydrophilic dyes a 20 cm column is preferable) for separation of protein from free dye. It is convenient to preequilibrate the column with phosphate buffered saline (PBS) or buffer of choice and to elute the protein using the same buffer. The first colored and fluorescent zone to elute will be the desired conjugate. A second colored and fluorescent, but slower moving zone contains the unlabeled free dye (hydrolyzed NHS-ester).

If the antibody solution to be conjugated is very dilute, to avoid further dilution you may want to purify the conjugate by extensive dialysis. However, dialysis does not yield as efficient and rapid separation as gel filtration. To prevent denaturation of the conjugate after elution, add bovine serum albumin (BSA) or any other stabilizer of choice to a final concentration of 1 - 10 mg/ml.

Storage of the Protein Conjugate

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 from light. We recommend to centrifuge conjugate solutions in a micro-centrifuge before use. This step will remove any aggregates that may have formed during long-term storage.

Determining the Degree of Labeling (DOL)

The degree of labeling (DOL, dye-to-protein ratio) obtained by the above procedure can be determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ϵ) × molar concentration × 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: $c(\text{dye}) = A_{\max} / \epsilon_{\max} \times d$, where $\epsilon_{\max}$ 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 $CF_{280} = \epsilon_{280} / \epsilon_{\max}$ are listed in the table on p.75. It follows for the absorbance of the protein itself:

$A_{\text{prot}} = A_{280} - A_{\max} \times CF_{280}$. Then the concentration of protein is:
 $c(\text{protein}) = A_{\text{prot}} / \epsilon_{\text{prot}} \times d$, where ϵ_{prot} is the extinction coefficient of the protein at 280 nm.

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

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

Note: The above relation is only valid if the extinction coefficient of the free dye $\epsilon_{\max}$ at the absorption maximum is the same as the extinction coefficient of the conjugated dye at this wavelength. Due to aggregation effects this is frequently not the case. Hence the value calculated for DOL may be too low by 20 % or more.

Labeling Proteins with Thiol-Reactive ATTO-Labels (Maleimides)

ATTO maleimides readily react with thiol groups of proteins. The optimum pH for the modification of thiols with maleimides is pH 7.0 - 7.5. We recommend the reaction to be carried out in phosphate buffered saline (PBS). At this pH the thiol group is sufficiently nucleophilic to react with the maleimide, whereas the amino groups of the protein show only little reactivity at this pH due to a high degree of protonation.

Procedure

Dissolve the protein at 50 - 100 μM in PBS at pH 7.0 - 7.5. Reduction of disulfide bonds in the protein can be achieved by adding a tenfold molar excess of dithiothreitol (DTT) or other reducing agent. If DTT is used as a reducing agent, the excess has to be removed by dialysis prior to addition of the maleimide. This may not be necessary with other reducing agents. We recommend to carry out the thiol modification in an inert atmosphere to prevent oxidation of the thiols. It may also be advisable to deoxygenate all buffers and solvents used for the thiol conjugation.

Prepare a 1 - 10 mM stock solution of the ATTO maleimide in anhydrous DMSO or DMF. Note that such solutions are not stable for a long period of time. Hence we recommend to freshly prepare the dye solutions immediately prior to use. Add a 10 - 20 fold molar excess of reactive dye to the protein solution whilst stirring and incubate 2 hours at room temperature.

To bind any remaining reactive dye add an excess of a low molecular weight thiol, e.g. glutathione or mercaptoethanol.

Separation of the Conjugate from Free Dye

To separate the dye labeled protein from unlabeled dye we recommend gel filtration using a Sephadex G-25 or equivalent gel filtration column (minimum of 1 cm diameter and 12 cm length; for very hydrophilic dyes a 20 cm column is preferable). Preequilibrate the column with phosphate buffered saline (PBS) or buffer of choice and elute the protein using the same buffer. The first colored and fluorescent zone to elute will be the dye labeled protein. A second colored and fluorescent, but slower moving zone contains the unlabeled free dye (hydrolyzed maleimide) and low-molecular-weight dye conjugate, i.e. the conjugate of excess dye maleimide with, e.g., mercaptoethanol.

Labeling Amine-Modified Oligonucleotides

The oligonucleotide must be functionalized with an amino group. The procedure given below is for labeling of an amine-modified oligonucleotide of 18 to 24 bases in length and is valid for oligonucleotides containing a single amino group. For the success of the conjugation reaction the purity of the oligonucleotide is very important. It must be free of primary and secondary amines. Especially contaminations such as Tris, glycine and ammonium salts can inhibit the reaction. We therefore strongly recommend purification by extraction and precipitation of the sample prior to labeling:

Purification of the Amine-Modified Oligonucleotide

- Dissolve 100 µg of the oligonucleotide in 100 µl demineralized water and extract three times with an equal amount of chloroform.
- Precipitate the oligonucleotide by adding 10 µl of 3 M sodium chloride and 250 µl of ethanol. Mix well and store at -20 °C for at least 30 minutes.
- Centrifuge the solution in a micro-centrifuge at about 12000 g for 30 minutes.
- Carefully remove the supernatant, rinse the pellet twice with small amounts of cold 70 % ethanol and dry under vacuum.
- Finally dissolve the dry pellet in demineralized water to a concentration of 25 µg / µl (4.2 mM for an 18-mer). This stock solution may be stored at -20 °C.

Recommended Buffers

- 0.1 M sodium carbonate buffer (pH 9):
Dissolve 0.5 nmol / µl sodium carbonate in demineralized water.
- 0.1 M sodium tetraborate buffer (pH 8.5):
Dissolve 0.038 g of sodium tetraborate decahydrate for every ml of demineralized water. Adjust pH with hydrochloric acid to 8.5.

Either one of these buffers should be prepared as close as possible to the start of the labeling procedure. Small aliquots may be frozen immediately for long term storage. Avoid exposure to air for a long time as the uptake of carbon dioxide will lower the pH of the buffer.

Procedure

Dissolve the amine-reactive dye in anhydrous amine-free DMF or DMSO to a concentration of 20 µg / µl. To 15 µl of this solution add 7 µl demineralized water, 75 µl buffer and 4 µl of 25 µg / µl stock solution of the amine-modified oligonucleotide. The reaction mixture may be a suspension rather than a clear solution. However, this does not affect the conjugation reaction. The mixture is stirred at room temperature for 3 to 6 hours. The yield of labeling will vary from 50 to 90 %. Longer incubation times do not necessarily result in greater labeling efficiency. Some loss of reactive dye is unavoidable due to hydrolysis of the NHS-ester.

Note: The reaction may be scaled up or down as long as the concentrations of the components are maintained. However, significant alteration of the relative concentrations of the components may drastically reduce the labeling efficiency.

Separation and Purification of the Conjugate

We recommend the precipitation of the oligonucleotide with ethanol as the first step of purification. To achieve this, add 10 µl of 3 M sodium chloride and 250 µl of ethanol to the reaction mixture. Proceed as described under *Purification of the amine-modified oligonucleotide*.

Note: Do not dry completely as the labeled oligonucleotide may become difficult to redissolve. The labeled oligonucleotide can be separated from unlabeled oligonucleotide and free dye by preparative gel electrophoresis or reversed-phase HPLC.

Purification by Gel Electrophoresis

For purification by gel electrophoresis use a 0.2 mm thick polyacrylamide slab gel with the following concentrations:

< 25 bases	19 % polyacrylamide
25 - 40 bases	15 % polyacrylamide
40 - 100 bases	12 % polyacrylamide

Suspend the residue from the ethanol precipitation in 200 μ l of 50 % formamide. Heat to 55 °C and incubate for 5 minutes. This will disrupt any secondary structure. Load the warmed sample onto the gel and load an adjacent well with 50 % formamide containing 0.05 % Bromophenol Blue as indicator. The indicator has approximately the same R_f value (will migrate with approximately the same rate) as the oligonucleotide. Run the gel until the Bromophenol Blue has reached two thirds of the way down the gel. Remove the gels from the plates and place on Saran Wrap™. Lay the gel on a fluorescent TLC plate. Illuminate with UV-light at 366 nm. The band which shows fluorescence is the labeled oligonucleotide. Cut out the fluorescent band and purify using the crush and soak method or other suitable techniques. For more details, please refer to Sambrook J., Fritsch E.F. and Maniatis, T., *Molecular cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbour Laboratory (1989).

Purification by HPLC

For HPLC purification you may use a standard analytical RP-C18 (4.6 x 250 mm) column. Dissolve the residue from the ethanol precipitation in 0.1 M TEAA (triethylammonium acetate), load onto the column and run a linear solvent gradient of 0 - 75 % acetonitrile in 0.1 M TEAA with an increase of acetonitrile of 2 % per minute. This gradient should be adjusted for very hydrophobic labeled oligonucleotides up to 3 % per minute. For more hydrophilic dyes you should run a slower gradient of about 1 % per minute. Usually the unlabeled oligonucleotide will migrate fastest followed by the labeled oligonucleotide and finally the free dye. For more details, please refer to Oliver R.W.A., *HPLC of Macromolecules: A Practical Approach*, IRL Press (1989).

Table: Optical properties of ATTO-labels

Dye	MW, g/mol NHS	Mal	λ_{abs} , nm	ϵ_{max} , M ⁻¹ cm ⁻¹	CF ₂₆₀	CF ₂₈₀
ATTO 390	440	465	390	2.4 x 10 ⁴	0.52	0.08
ATTO 425	498	523	436	4.5 x 10 ⁴	0.27	0.23
ATTO 465	493	518	453	7.5 x 10 ⁴	1.12	0.54
ATTO 488	981	1067	501	9.0 x 10 ⁴	0.25	0.10
ATTO 495	549	574	495	8.0 x 10 ⁴	0.57	0.39
ATTO 520	564	589	516	1.1 x 10 ⁵	0.40	0.40
ATTO 532	1081	1063	532	1.15 x 10 ⁵	0.22	0.11
ATTO Rho6G	711	849	535	1.15 x 10 ⁵	0.22	0.19
ATTO 540Q	756	781	542	1.05 x 10 ⁵	0.22	0.24
ATTO 550	791	816	554	1.2 x 10 ⁵	0.24	0.12
ATTO 565	708	733	563	1.2 x 10 ⁵	0.34	0.16
ATTO Rho3B	642	764	565	1.2 x 10 ⁵	0.28	0.14
ATTO Rho11	763	788	571	1.2 x 10 ⁵	0.25	0.09
ATTO Rho12	847	872	576	1.2 x 10 ⁵	0.27	0.09
ATTO Thio12	699	824	579	1.1 x 10 ⁵	0.10	0.37
ATTO Rho101	787	812	586	1.2 x 10 ⁵	0.24	0.19
ATTO 580Q	892	917	586	1.1 x 10 ⁵	0.36	0.13
ATTO 590	788	813	594	1.2 x 10 ⁵	0.42	0.44
ATTO Rho13	843	867	600	1.2 x 10 ⁵	0.38	0.44
ATTO 594	1389	1358	601	1.2 x 10 ⁵	0.26	0.51
ATTO 610	588	613	615	1.5 x 10 ⁵	0.02	0.05
ATTO 611X	604	629	611	1.0 x 10 ⁵	0.05	0.07
ATTO 612Q	888	913	615	1.15 x 10 ⁵	0.35	0.57
ATTO 620	709	734	619	1.2 x 10 ⁵	0.05	0.07
ATTO Rho14	981	1019	625	1.4 x 10 ⁵	0.29	0.46
ATTO 633	749	774	629	1.3 x 10 ⁵	0.05	0.06
ATTO 647	811	832	645	1.2 x 10 ⁵	0.08	0.04
ATTO 647N	843	868	644	1.5 x 10 ⁵	0.06	0.05
ATTO 655	887	812	663	1.25 x 10 ⁵	0.24	0.08
ATTO Oxa12	835	874	663	1.25 x 10 ⁵	0.24	0.08
ATTO 665	820	845	663	1.60 x 10 ⁵	0.07	0.06
ATTO 680	828	1024	680	1.25 x 10 ⁵	0.30	0.17
ATTO 700	837	971	700	1.2 x 10 ⁵	0.26	0.41
ATTO 725	613	638	729	1.2 x 10 ⁵	0.10	0.08
ATTO 740	665	690	740	1.2 x 10 ⁵	0.11	0.10
ATTO MB2	553	591	658	1.0 x 10 ⁵	0.11	0.28

In cooperation with Jena Bioscience **ATTO-TEC** offers fluorescence-labeled nucleotides.

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)

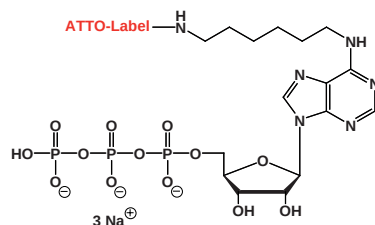
All labeled nucleotides are supplied as ready-to-use **0.5** or **1.0 mM** aqueous solutions in units of 10, 20, 30, 40 or 50 μ l depending on the particular nucleotide and/or label.

To create the applicable order code, please replace the xxx in the order codes below by the **ATTO**-dye number.

Labeled Adenosine Nucleotides

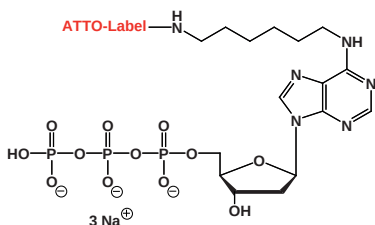
N⁶-(6-Amino)hexyl-ATP

Order code:
NU-805-xxx



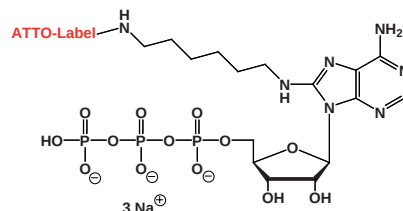
N⁶-(6-Amino)hexyl-dATP

Order code:
NU-835-xxx



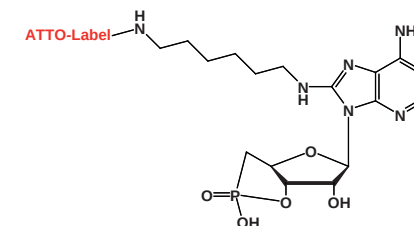
8-[(6-Amino)hexyl]-amino-ATP

Order code:
NU-807-xxx



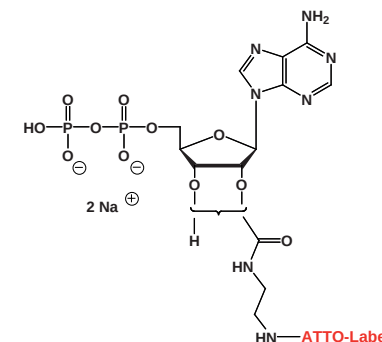
8-[(6-Amino)hexyl]-amino-cAMP

Order code:
NU-851-xxx



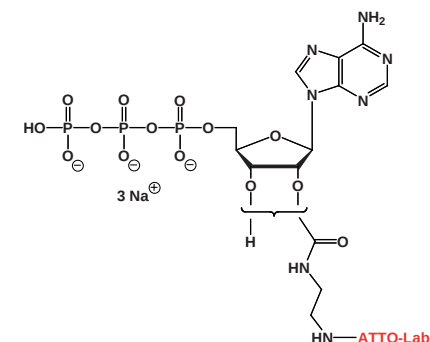
EDA-ADP

Order code:
NU-802-xxx



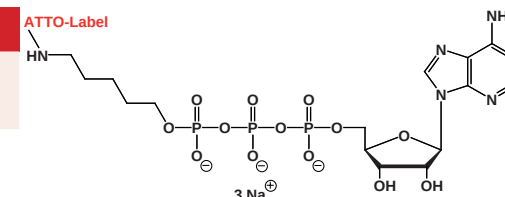
EDA-ATP

Order code:
NU-808-xxx



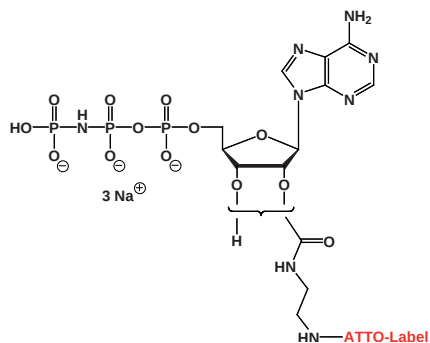
γ -(6-Aminohexyl)-ATP

Order code:
NU-833-xxx



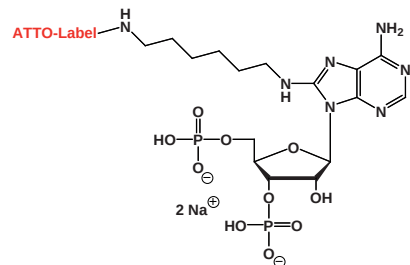
EDA-AppNHp (EDA-AMPPNP)

Order code:
NU-810-xxx



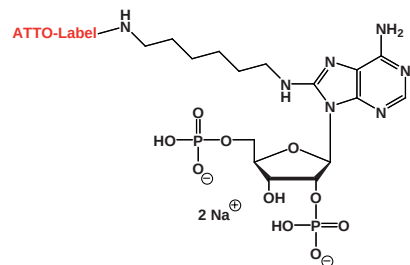
8-[(6-Amino)hexyl]-amino-adenosine-3',5'-bisphosphate

Order code:
NU-811-xxx



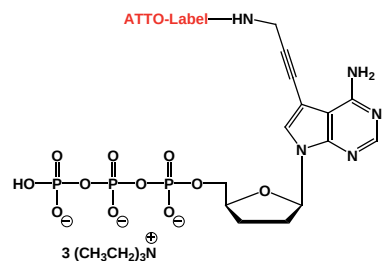
8-[(6-Amino)hexyl]-amino-adenosine-2',5'-bisphosphate

Order code:
NU-812-xxx



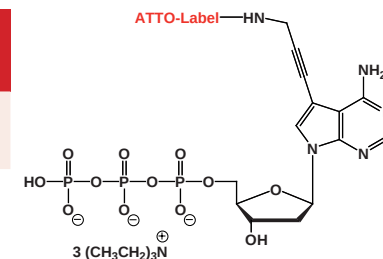
7-Propargylamino-7-deaza-ddATP

Order code:
NU-1612-xxx



7-Propargylamino-7-deaza-dATP

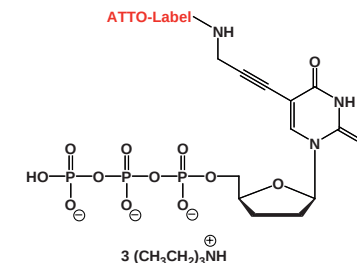
Order code:
NU-1611-xxx



Labeled Cytidine Nucleotides

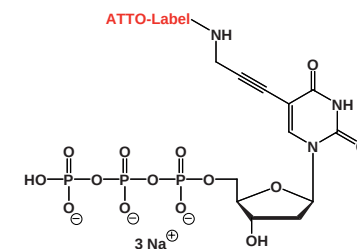
5-Propargylamino-ddCTP

Order code:
NU-850-xxx



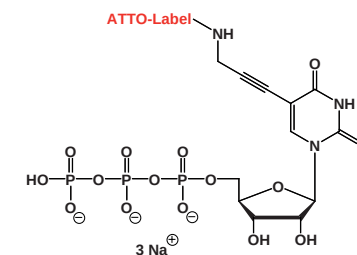
5-Propargylamino-dCTP

Order code:
NU-809-xxx



5-Propargylamino-CTP

Order code:
NU-831-xxx

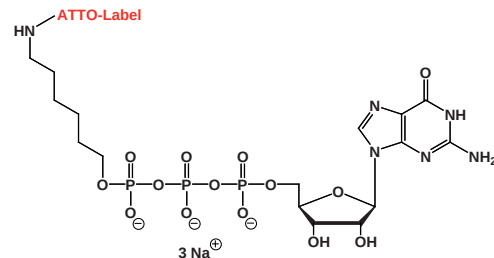


Labeled Guanosine and m⁷ Guanosine Nucleotides

γ-(6-Aminohexyl)-GTP

Order code:

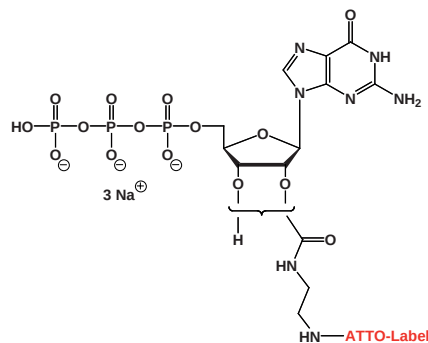
NU-834-xxx



EDA-GTP

Order code:

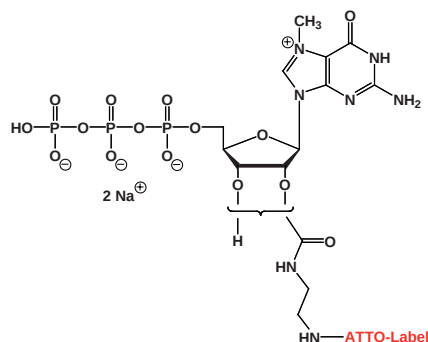
NU-820-xxx



EDA-m⁷-GTP

Order code:

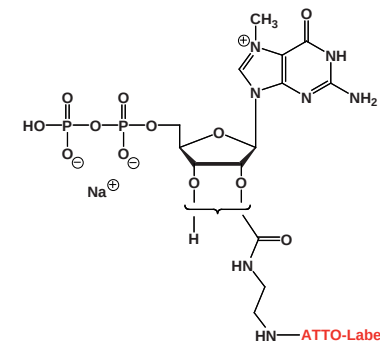
NU-824-xxx



EDA-m⁷-GDP

Order code:

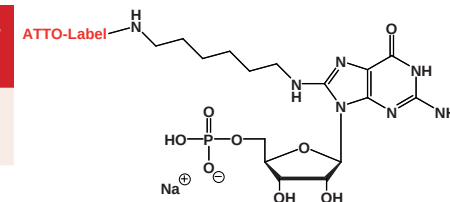
NU-827-xxx



8-[(6-Amino)hexyl]-amino-GMP

Order code:

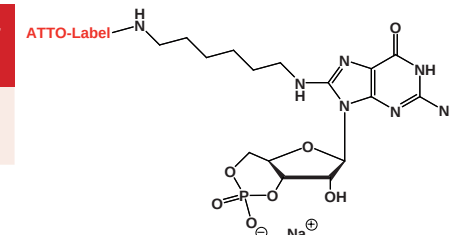
NU-829-xxx



8-[(6-Amino)hexyl]-amino-cGMP

Order code:

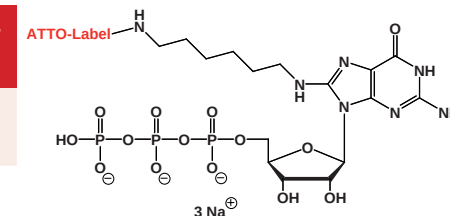
NU-832-xxx



8-[(6-Amino)hexyl]-amino-GTP

Order code:

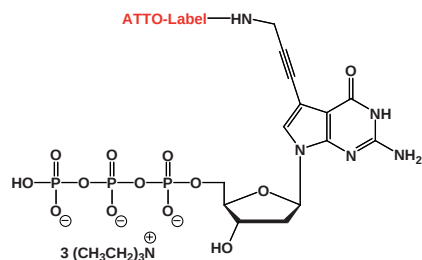
NU-830-xxx



7-Propargylamino-7-deaza-dGTP

Order code:

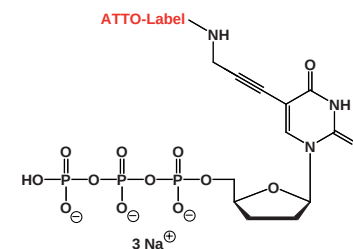
NU-1615-xxx



5-Propargylamino-ddUTP

Order code:

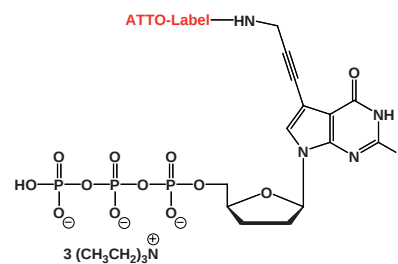
NU-1619-xxx



7-Propargylamino-7-deaza-ddGTP

Order code:

NU-1618-xxx



Labeled ATP γ S and GTP γ S Nucleotides

EDA-ATP γ S

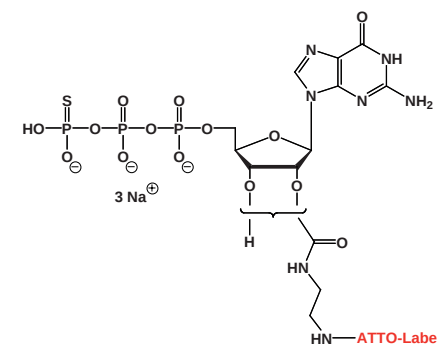
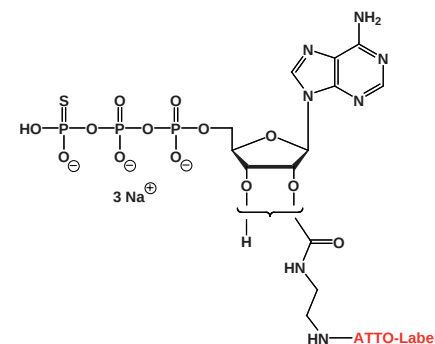
Order code:

NU-1609-xxx

EDA-GTP γ S

Order code:

NU-1610-xxx

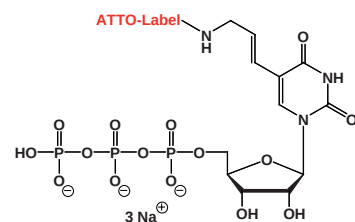


Labeled Uridine Nucleotides

Aminoallyl-UTP

Order code:

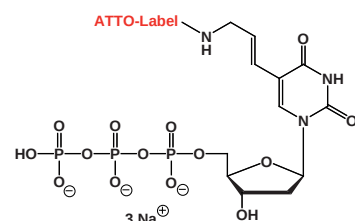
NU-821-xxx



Aminoallyl-dUTP

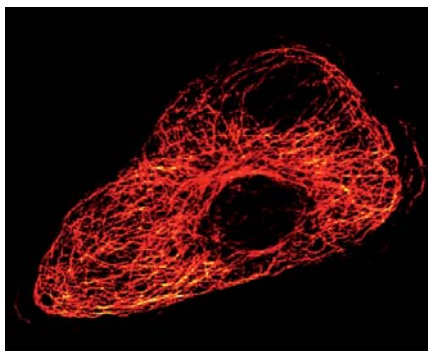
Order code:

NU-803-xxx

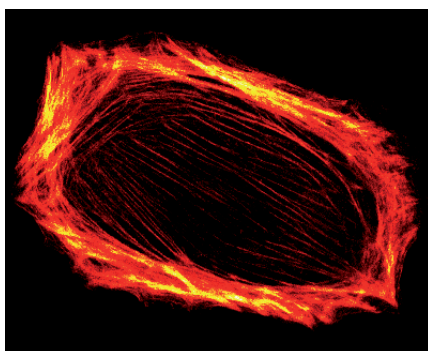


ATTO-Labels:

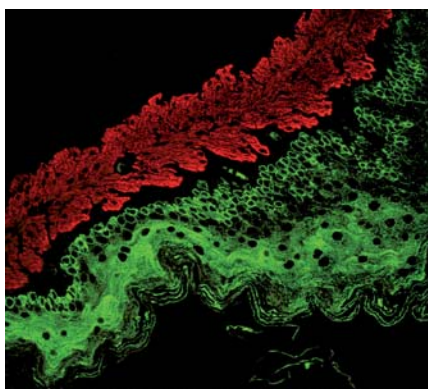
ATTO 390	ATTO 425	ATTO 465	ATTO 488	ATTO 495
ATTO 532	ATTO Rho 6G	ATTO 540Q	ATTO 550	ATTO 565
ATTO Rho3B	ATTO Rho11	ATTO Rho12	ATTO Thio12	ATTO 580Q
ATTO Rho101	ATTO 590	ATTO 594	ATTO Rho13	ATTO 612Q
ATTO 620	ATTO Rho14	ATTO 633	ATTO 647N	ATTO 655
ATTO Oxa12	ATTO 665	ATTO 680	ATTO 700	ATTO 725
ATTO 740	ATTO MB2			



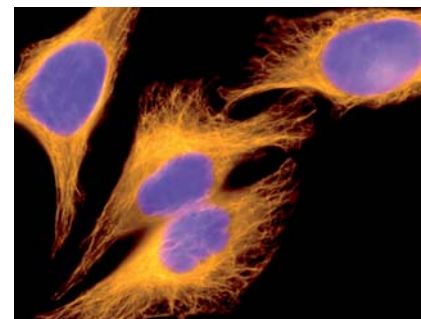
Tubulin (PtK2 - Male Rat Kangaroo Kidney Epithelial Cells)
Tubulin mouse IgG primary antibody bound to tubulin. Immunostaining with **ATTO 532** labeled sheep anti-mouse IgG secondary antibody.



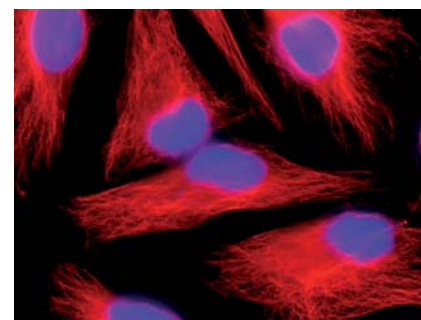
Actin (PtK2 - Male Rat Kangaroo Kidney Epithelial Cells)
ATTO 532 labeled phalloidin bound to actin.



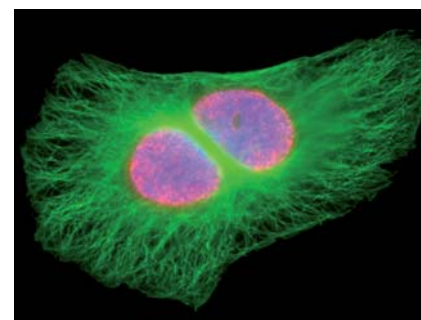
Rat stomach: Actin stained with mouse anti-smooth muscle α -actin antibody and **ATTO 488** anti-mouse IgG (green). Cytokeratin stained with polyclonal rabbit anti-cytokeratin and **ATTO 647N** anti-rabbit IgG (red).



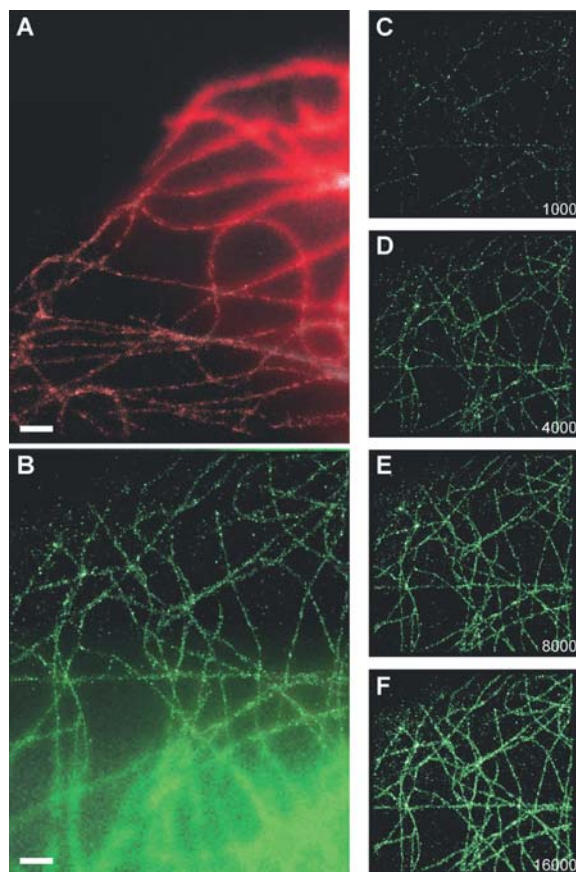
HeLa cells stained with LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Vendikar Yellow **ATTO 550** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.



HeLa cells stained with LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Regulus Red **ATTO 594** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.

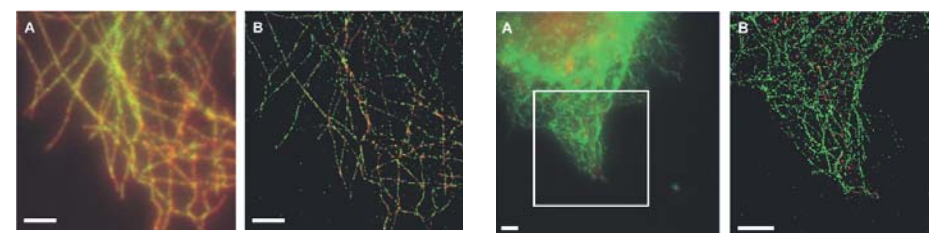


HeLa cells stained with LP Bio anti-trimethyl-Lys27 Histone H3 primary and the Regulus Red **ATTO 594** anti-rabbit secondary. Green: LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Regulus Red **ATTO 594** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.



Photoswitching microscopy performed with the two standard fluorophores **ATTO 655** and **ATTO 520**. The upper right (A) and lower (B) parts of the images 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 mercaptoethylamine 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 superresolution image with **ATTO 520** as fluorophore (1000-16000 frames corresponding to measurement times of 100-1600 s)

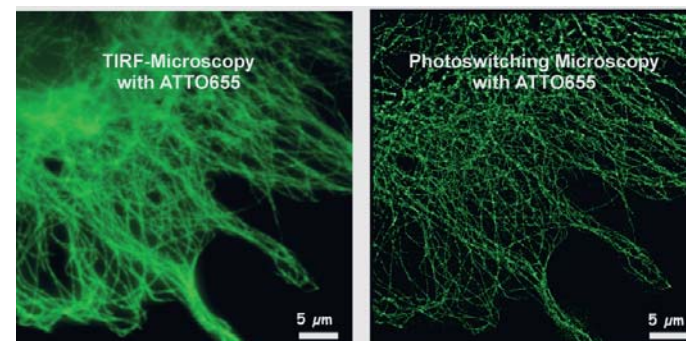
M. Sauer et al., Bielefeld University.



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 mercaptoethylamine 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).

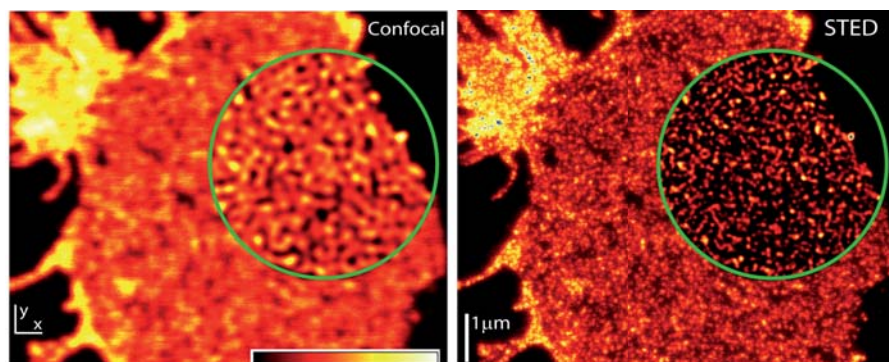
Dual-color photoswitching microscopy with **ATTO 520** labeled microtubules and **ATTO 655** labeled cytochrome c oxidase localized in the inner mitochondrial membrane of COS-7 cells. The reconstructed dual-color photoswitching image (expanded section) is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed successively in PBS, pH 7.4 in the presence of 100 mM mercaptoethylamine 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., Bielefeld University.



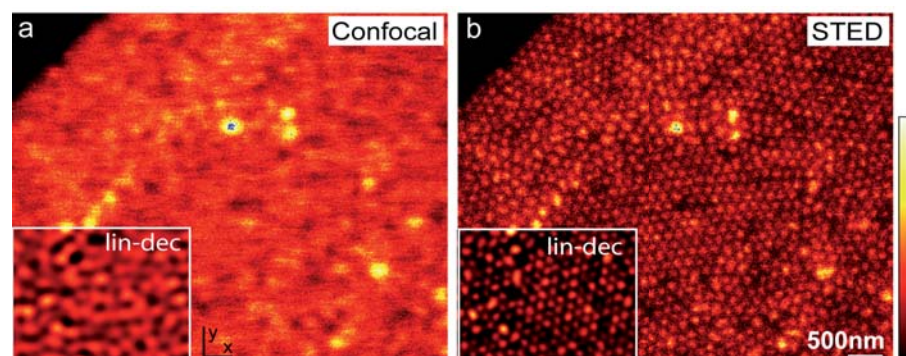
Photoswitching microscopy performed with **ATTO 655**. The left side shows a conventional immuno-fluorescence image of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** labeled F(ab')₂ fragments. The right side shows photoswitching microscopy image with subdiffraction resolution (scale bar 5 μm). Measurements were performed in PBS, pH 7.4 in the presence of 100 mM mercaptoethylamine at a laser power of 30 mW at 647 nm with a frame rate of 10 Hz.

M. Sauer et al., Bielefeld University.



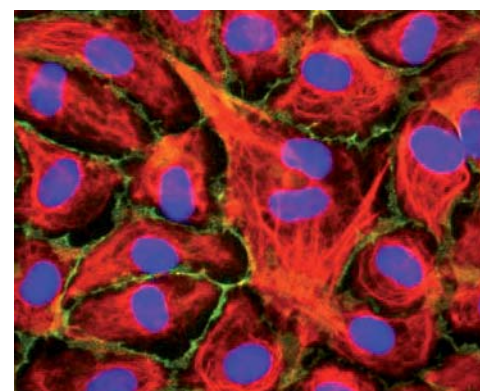
Imaging the spatial order of colloidal nanoparticles (a) Confocal image, (b) corresponding STED image. The silica nanoparticles feature a **ATTO 532** fluorescent core and a non-fluorescent shell. Only the STED image (b) reveals grain boundaries, defects and dislocations in the semi-crystalline nanoparticle formation.

S.W. Hell et al., MPI Göttingen.

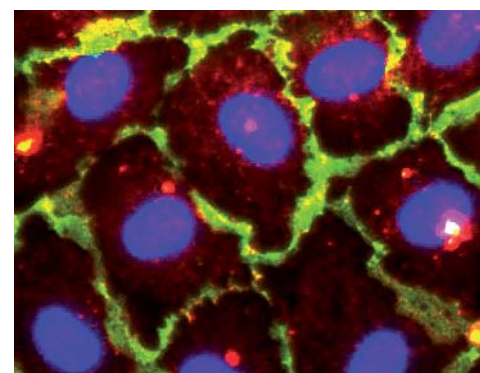


Revealing the nanopattern of the SNARE protein SNAP-25 on the plasma membrane of a mammalian cell. Confocal versus STED image of the antibody-tagged proteins. The secondary antibody was labeled with **ATTO 532-NHS**. The encircled areas show linearly deconvolved data. STED microscopy provides a substantial leap forward in the imaging of protein self-assembly.

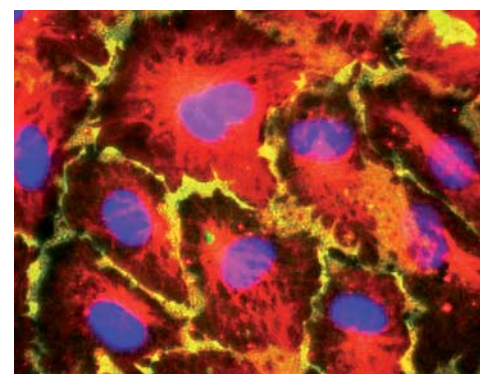
S.W. Hell et al., MPI Göttingen.



HUVEC: Vimentin/**ATTO 532**;
E-Cadherin/**ATTO 655** and DAPI



HUVEC: Inhibitor apoptosis protein/
ATTO 550; E-Cadherin/**ATTO 655**
and DAPI



HUVEC: alpha-Tubulin/**ATTO 532**;
E-Cadherin/**ATTO 655** and DAPI

Abbreviation	
λ	wavelength
λ_{abs}	longest-wavelength absorption maximum
ϵ_{max}	molar extinction coefficient at the longest-wavelength absorption maximum
ϵ_{260}	molar extinction coefficient at $\lambda = 260$ nm
ϵ_{280}	molar extinction coefficient at $\lambda = 280$ nm
CF_{260}	$\text{CF}_{260} = \epsilon_{260}/\epsilon_{\text{max}}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-DNA conjugates.
CF_{280}	$\text{CF}_{280} = \epsilon_{280}/\epsilon_{\text{max}}$. 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}	fluorescence decay time
τ_0	natural (radiative) decay time
MW	molecular weight
M^+	molecular weight of dye cation (HPLC-MS)
MH^+	molecular weight of protonated dye (HPLC-MS)
PBS	phosphate buffered saline
DOL	degree of labeling
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

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

- Prof. Dr. Stefan Hell and co-workers, Department of NanoBiophotonics, MPI for Biophysical Chemistry, Göttingen, Germany
- Prof. Dr. Markus Sauer, Applied Laser Physics & Laser Spectroscopy and Bielefeld Institute for Biophysics and Nanoscience (BINAS), Bielefeld University, Germany
- Sigma-Aldrich Production GmbH, Buchs, Switzerland
- Prof. Dr. Peter Friedl and co-workers, Department of Organic Chemistry and Biochemistry, TU Darmstadt, Germany
- Active Motif Corporation, Carlsbad, CA 92008, USA

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ATTO-TEC GmbH

Am Eichenhang 50
D-57076 Siegen
Germany

Phone: +49(0)-271-2 38 53-0

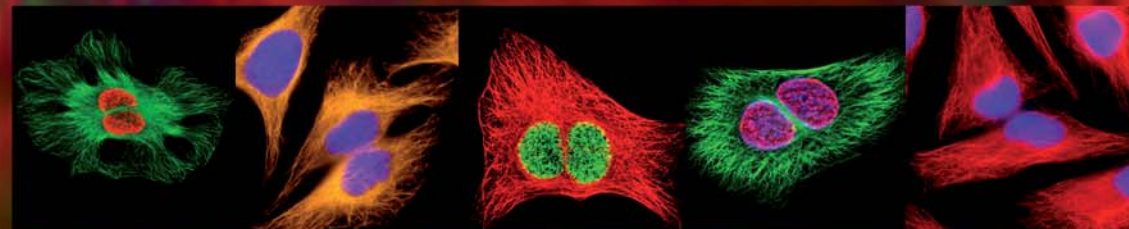
Fax: +49(0)-271-2 38 53-11

E-mail: sales@atto-tec.com

[http: www.atto-tec.com](http://www.atto-tec.com)

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