Fluorescent Labels and Dyes

catalogue 2007/2008

Cover:

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

ATTO-TEC

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Contents

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 are used now 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

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Fluorescence is Our Business

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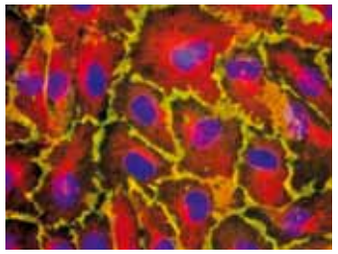
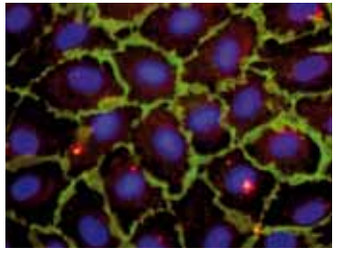
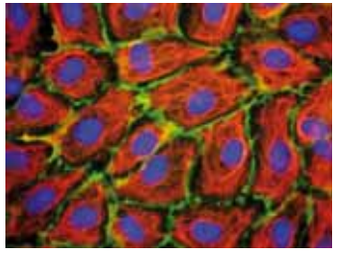


HUVEC: alpha-Tubulin/ATTO 532; E-Cadherin/ATTO 655 and DAPI



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



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

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 custom made filter set with high transmittance between 650 nm and 750 nm, ATTO 647**N** would be a very good choice. As can be seen from the list of ATTO-labels in this catalogue, ATTO 647**N** (p. 40) has a high extinction coefficient at 635 nm (as follows from ε_{max} and inspection of the absorption curve) as well as an excellent quantum yield of fluorescence (η_{fl} = 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 applied excitation source, a label with a slightly longer wavelength should be chosen. The absorbance will not decrease drastically, but the larger shift between excitation wavelength and fluorescence (the latter being independent of excitation wavelength with all dyes) provides the additional advantage of better discrimination against scattered excitation light.

Introduction

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 594, ATTO 610, ATTO 611X
Argon-Ion laser	488 nm	ATTO 488, ATTO 520 ATTO
	514 nm	520, ATTO 532, ATTO 550
Nd:YAG laser	532 nm	ATTO 532, ATTO 550, ATTO 565
He-Ne laser	633 nm	ATTO 633, ATTO 635, ATTO 637, ATTO 647, ATTO 647**N**
Krypton-Ion laser	647 nm	ATTO 647, ATTO 647**N**, ATTO 655, ATTO 680
	676 nm	ATTO 680, ATTO 700, ATTO 725, ATTO 740
Diode laser	635 nm	ATTO 635, ATTO 637, ATTO 647, ATTO 647**N**, 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
$\kappa^2 = (\cos\varphi_{DA} - 3\cos\varphi_D \cos\varphi_A)^2$
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. 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 important to take the relative orientation of donor and acceptor into account.

Introduction

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

Donor **Acceptor**

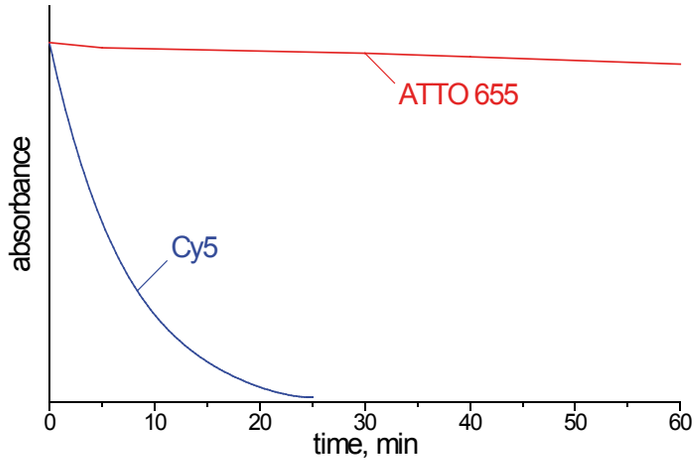
	390	425	465	488	495	520	532	540Q	550	565	580Q	590	594	610
390	14	41	50	58	59	60	56	54	53	52	47	48	45	44
425		36	46	59	59	61	58	56	56	55	51	51	49	48
465			35	52	51	61	56	55	56	55	53	54	52	52
488				50	46	61	64	63	63	63	60	60	57	57
495					41	56	56	56	57	58	55	56	54	54
520						57	65	66	67	67	64	64	61	60
532							57	63	68	68	67	68	66	66
550									58	63	69	70	68	69
565										61	69	72	71	73
590												63	66	73
594													62	70
610														64
611X														
620														
633														
635														
637														
647														
647N														
655														
680														
700														
725														
740														

	611X	612Q	620	633	635	637	647	647N	655	680	700	725	740
390	40	43	41	41	39	40	39	39	40	38	35	36	36
425	44	47	45	45	43	44	43	43	43	41	38	36	37
465	49	52	49	49	48	49	48	48	48	46	43	41	40
488	53	55	53	53	52	53	51	51	50	48	44	41	40
495	51	53	51	51	50	50	49	49	49	47	44	42	41
520	56	59	56	55	55	56	54	53	53	50	46	43	41
532	62	64	61	61	61	62	60	59	59	57	53	50	48
550	65	67	67	66	66	67	65	65	64	62	58	55	53
565	69	70	69	69	69	70	69	68	68	65	61	58	56
590	69	71	71	73	73	73	73	74	73	71	69	66	63
594	67	68	70	73	73	73	74	75	75	74	72	69	68
610	64	63	66	70	71	73	72	73	76	75	74	69	68
611X	44	44	45	50	52	52	57	56	62	65	66	65	64
620			58	64	66	65	68	70	70	69	68	67	65
633				60	63	62	68	69	72	73	72	72	71
635					54	53	58	59	63	63	62	62	61
637						51	56	58	61	62	62	62	61
647							51	52	58	60	61	61	60
647N								65	72	75	74	73	72
655									58	64	66	66	65
680										59	65	67	66
700											58	66	66
725												52	56
740													54

Introduction

Properties of Fluorescent Labels

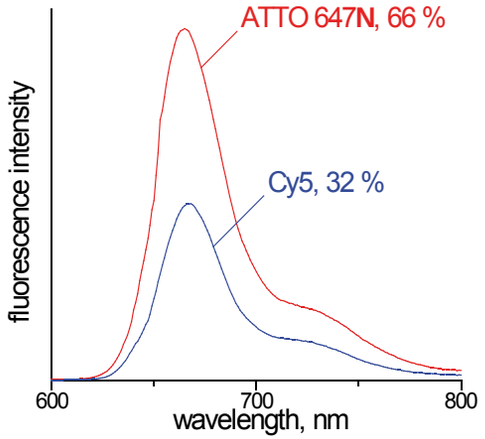
Apart from absorption and fluorescence there are many other dye properties that are highly relevant with respect to their suitability as labels. Most important, the dye must remain intact during irradiation. Many common labels, e.g. Fluorescein (FITC), show very low photostability. As a result sensitivity 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 Cy5™ in water. Irradiation with a 250 W tungsten-halogen lamp. Absorbance vs. time of illumination.

Many common fluorescent labels deteriorate even without any irradiation (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 Cy5™ and Alexa 647™. 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 can be used in place of gas lasers. Diode lasers are generally less expensive and more energy-efficient. Furthermore a variety of sensitive detectors is now 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 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 old Cy5™.



Fluorescence quantum yield of **ATTO 647N** compared with common Cy5™ in water. Solutions of equal absorbance excited at 647 nm. Fluorescence intensity vs. wavelength.

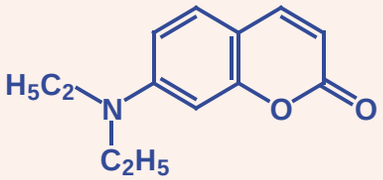
Introduction

Structure of Fluorescent Labels

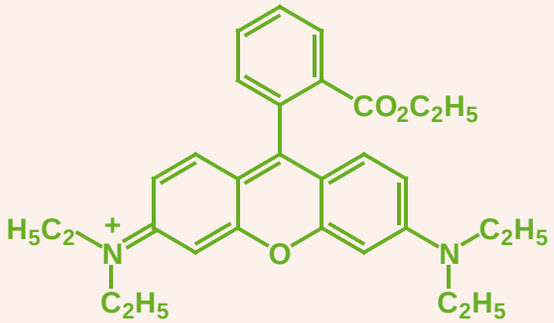
The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of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

ATTO-labels are typically derivatives of:

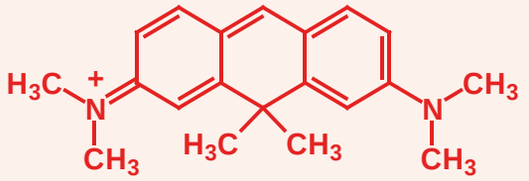
- **Coumarin**



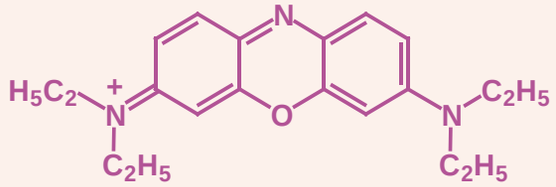
- **Rhodamine**



- **Carbopyronin**



- **Oxazine**



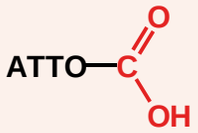
Reactive Labels and Conjugates

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. Dyes with other reactive substituents can be supplied on request.

Furthermore ATTO-dyes conjugated to streptavidin and other proteins are available on request. The high affinity of streptavidin to biotin is the basis for the wide-spread use of streptavidin conjugates. In this connection all ATTO-dyes are also offered as biotin conjugates.

ATTO-dye with free COOH:



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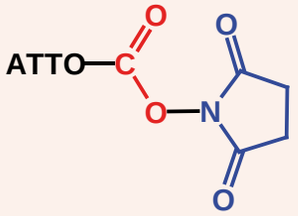
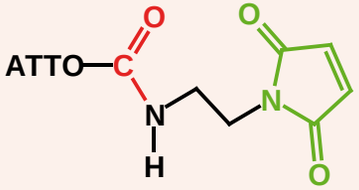
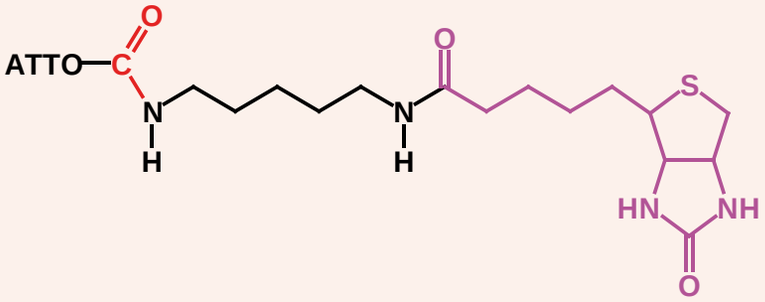


maleimide:



biotin derivative:



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*, *Documents*, *Spectral data*). - The correction factors CF_{260} and CF_{280} aide with calculating the degree of labeling (DOL), see „Labeling Procedures“ (p. 56-61).

The molecular weight (*MW*) given has the common meaning, i.e. it refers to the dye including counterions. For mass spectrometry purposes also the mass of the single-charged ion (M^+ or MH^+) is given.

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

Fluorescent Labels

ATTO Fluorescent Labels

Label	λ_{abs}, nm	ε_{max}, $M^{-1}\,cm^{-1}$	λ_{fl}, nm	η_{fl}, %	τ_{fl}, ns	Alternative to
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 550	554	120000	576	80	3.2	TAMRA**, Cy3***
ATTO 565	563	120000	592	90	3.4	Cy3.5***, ROX**
ATTO 590	594	120000	624	80	3.7	Alexa 594*, Texas Red*
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 633	629	130000	657	64	3.2	Alexa 633*
ATTO 635	635	120000	659	25	1.9	Alexa 633*
ATTO 637	635	120000	659	25	1.9	Alexa 633*
ATTO 647	645	120000	669	20	2.3	Cy5***, Alexa 647*
ATTO 647**N**	644	150000	669	65	3.4	Cy5***, Alexa 647*
ATTO 655	663	125000	684	30	1.9	Cy5***, Alexa 647*
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	

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

ATTO Fluorescence Quenchers (p. 46-49)

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

Dyes with Large Stokes-Shift

Label	λ_{abs}, nm	ε_{max}, $M^{-1}\,cm^{-1}$	λ_{fl}, nm	η_{fl}, %	τ_{fl}, ns
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 611X	611	100000	681	35	2.5

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}	$CF_{260} = \varepsilon_{260}/\varepsilon_{max}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-DNA conjugates.
CF_{280}	$CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-protein conjugates.

ATTO 390

Solvent: **Water**

λ_{abs} = 390 nm

ε_{max} = 2.4 x 10^4 M^{-1} cm^{-1}

λ_{fl} = 479 nm

η_{fl} = 90 %

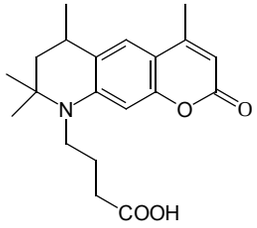
τ_{fl} = 3.8 ns

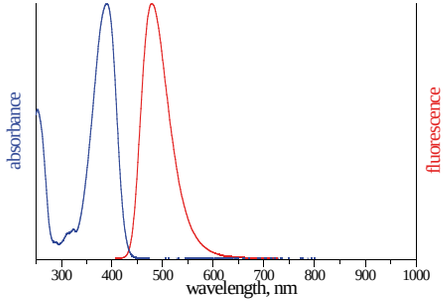
CF_{260} = 0.52

CF_{280} = 0.08

Features:

- High fluorescence yield
- Large Stokes-shift
- Moderately hydrophilic
- Good solubility in polar solvents
- Coumarin derivative, uncharged





Modification	MW, g/mol	MH^+, g/mol	Unit	Order Code
with free COOH	343.4	344	1 mg 5 mg	AD 390-21 AD 390-25
NHS-ester	440.5	441	1 mg 5 mg	AD 390-31 AD 390-35
maleimide	465.6	466	1 mg 5 mg	AD 390-41 AD 390-45
biotin derivative	653.9	654	1 mg 5 mg	AD 390-71 AD 390-75

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

Fluorescent Labels

ATTO 425

Solvent: **Water**

λ_{abs} = 436 nm

ε_{max} = 4.5 x 10^4 M^{-1} cm^{-1}

λ_{fl} = 484 nm

η_{fl} = 90 %

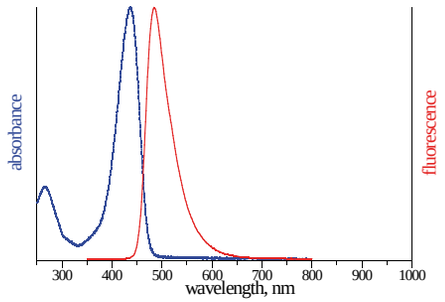
τ_{fl} = 3.5 ns

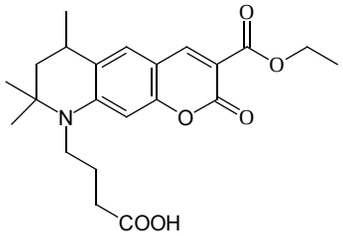
CF_{260} = 0.27

CF_{280} = 0.23

Features:

- High fluorescence yield
- Large Stokes-shift
- Moderately hydrophilic
- Good solubility in polar solvents
- Coumarin derivative, uncharged





Modification	MW, g/mol	MH^+, g/mol	Unit	Order Code
with free COOH	401.5	402	1 mg 5 mg	AD 425-21 AD 425-25
NHS-ester	498.5	499	1 mg 5 mg	AD 425-31 AD 425-35
maleimide	523.6	524	1 mg 5 mg	AD 425-41 AD 425-45
biotin derivative	711.9	712	1 mg 5 mg	AD-425-71 AD-425-75

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

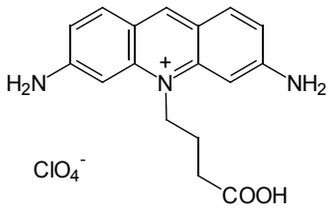
ATTO 465

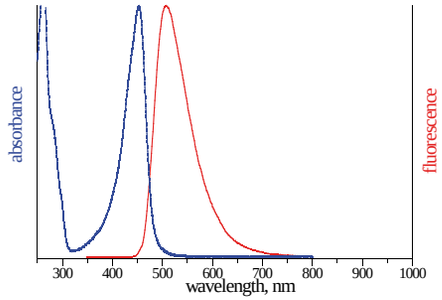
Solvent: **Water**

λ_{abs}	=	453 nm			
ε_{max}	=	7.5 x 10^4 M^{-1} cm^{-1}			
λ_{fl}	=	508 nm			
η_{fl}	=	55 %	CF_{260}	=	1.12
τ_{fl}	=	2.2 ns	CF_{280}	=	0.54

Features:

- High fluorescence yield
- Large Stokes-shift in aqueous solution
- High triplet yield, intense phosphorescence in solid matrix
- Hydrophilic
- Good solubility in all polar solvents
- Cationic dye derived from well-known Acriflavine, perchlorate salt





Modification	MW, g/mol	M⁺, g/mol	Unit	Order Code
with free COOH	395.8	296	1 mg 5 mg	AD 465-21 AD 465-25
NHS-ester	492.9	393	1 mg 5 mg	AD 465-31 AD 465-35
maleimide	517.9	418	1 mg 5 mg	AD 465-41 AD 465-45
biotin derivative	706.3	606	1 mg 5 mg	AD 465-71 AD 465-75

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

Fluorescent Labels

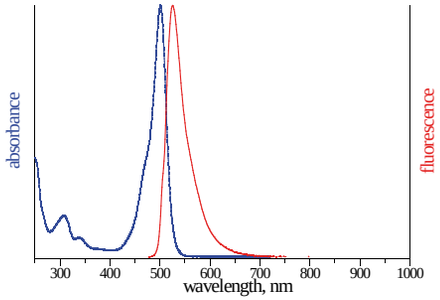
ATTO 488

Solvent: **Water**

λ_{abs}	=	501 nm			
ε_{max}	=	9.0 x 10^4 M^{-1} cm^{-1}			
λ_{fl}	=	523 nm			
η_{fl}	=	80 %	CF_{260}	=	0.25
τ_{fl}	=	3.2 ns	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	Unit	Order Code
with free COOH	804	590	1 mg 5 mg	AD 488-21 AD 488-25
NHS-ester	981	687	1 mg 5 mg	AD 488-31 AD 488-35
maleimide	1067	712	1 mg 5 mg	AD 488-41 AD 488-45
biotin derivative	1191	900	1 mg 5 mg	AD 488-71 AD 488-75

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

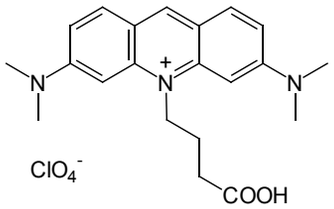
ATTO 495

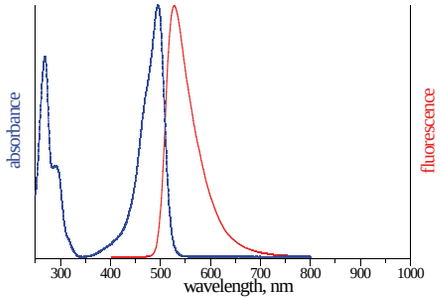
Solvent: **Water**

λ_{abs}	=	495 nm			
ε_{max}	=	8.0 x 10^4 M^{-1} cm^{-1}			
λ_{fl}	=	527 nm			
η_{fl}	=	45 %	CF_{260}	=	0.57
τ_{fl}	=	2.4 ns	CF_{280}	=	0.39

Features:

- High triplet yield
- Phosphorescent in solid matrix
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye derived from well-known Acridine Orange, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	451.9	352	1 mg 5 mg	AD 495-21 AD 495-25
NHS-ester	549.0	449	1 mg 5 mg	AD 495-31 AD 495-35
maleimide	574.0	474	1 mg 5 mg	AD 495-41 AD 495-45
biotin derivative	762.4	662	1 mg 5 mg	AD 495-71 AD 495-75

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

Fluorescent Labels

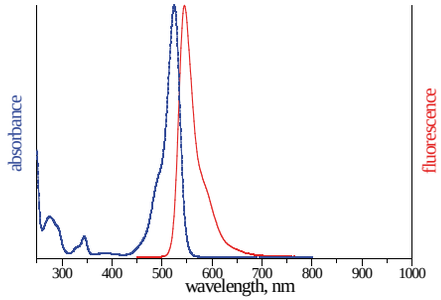
ATTO 520

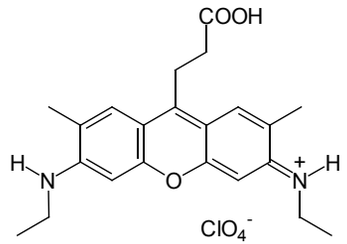
Solvent: **Water**

λ_{abs}	=	516 nm			
ε_{max}	=	1.1 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	538 nm			
η_{fl}	=	90 %	CF_{260}	=	0.40
τ_{fl}	=	3.8 ns	CF_{280}	=	0.40

Features:

- High fluorescence yield
- High thermal and photostability
- Moderately hydrophilic
- Good solubility in all polar solvents
- At pH > 7 reversible formation of colorless pseudobase
- Cationic dye closely related to well-known Rhodamine 6G, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	466.9	367	1 mg 5 mg	AD 520-21 AD 520-25
NHS-ester	564.0	464	1 mg 5 mg	AD 520-31 AD 520-35
maleimide	589,0	489	1 mg 5 mg	AD 520-41 AD 520-45
biotin derivative	777.4	677	1 mg 5 mg	AD 520-71 AD 520-75

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

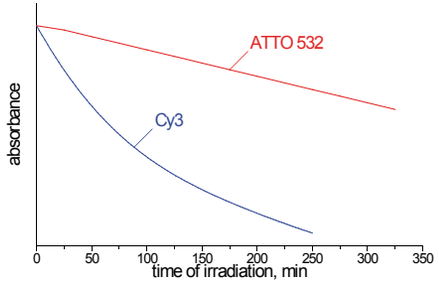
ATTO 532

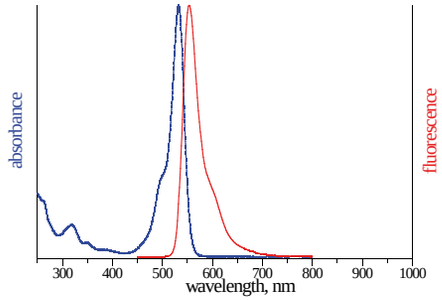
Solvent: **Water**

λ_{abs}	=	532 nm			
ε_{max}	=	1.15 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	553 nm			
η_{fl}	=	90 %	CF_{260}	=	0.22
τ_{fl}	=	3.8 ns	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	Unit	Order Code
with free COOH	765	646	1 mg 5 mg	AD 532-21 AD 532-25
NHS-ester	1081	743	1 mg 5 mg	AD 532-31 AD 532-35
maleimide	1063	768	1 mg 5 mg	AD 532-41 AD 532-45
biotin derivative	1357	956	1 mg 5 mg	AD 532-71 AD 532-75

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

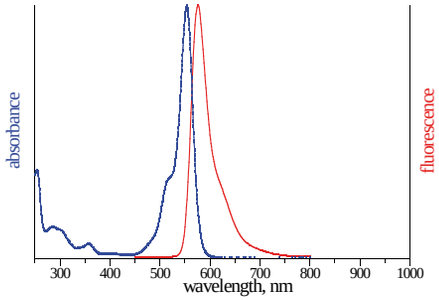
ATTO 550

Solvent: **Water**

λ_{abs}	=	554 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	576 nm			
η_{fl}	=	80 %	CF_{260}	=	0.24
τ_{fl}	=	3.2 ns	CF_{280}	=	0.12

Features:

- High fluorescence yield
- High thermal and photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye closely related to well-known Rhodamine 6G, perchlorate salt
- Supplied as mixture of three diastereomers



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	694.2	594	1 mg 5 mg	AD 550-21 AD 550-25
NHS-ester	791.3	691	1 mg 5 mg	AD 550-31 AD 550-35
maleimide	816.4	716	1 mg 5 mg	AD 550-41 AD 550-45
biotin derivative	1004.7	904	1 mg 5 mg	AD 550-71 AD 550-75

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

ATTO 565

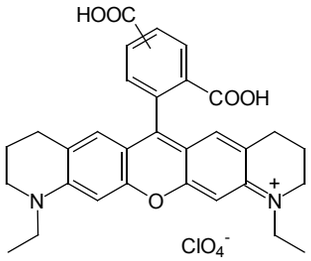
Solvent: **Water**

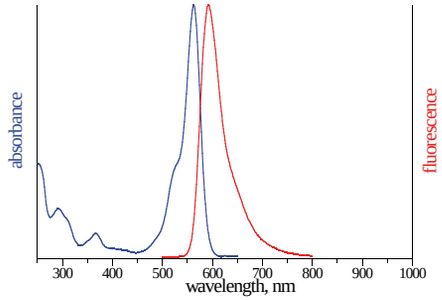
λ_{abs} = 563 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 592 nm
η_{fl} = 90 %
τ_{fl} = 3.4 ns

CF_{260} = 0.34
CF_{280} = 0.16

Features:

- High fluorescence yield
- High thermal and photostability
- Good solubility in polar solvents
- Rhodamine dye related to well-known Rhodamine 101, perchlorate salt
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	611.0	511	1 mg 5 mg	AD 565-21 AD 565-25
NHS-ester	708.1	608	1 mg 5 mg	AD 565-31 AD 565-35
maleimide	733.2	633	1 mg 5 mg	AD 565-41 AD 565-45
biotin derivative	921.5	821	1 mg 5 mg	AD 565-71 AD 565-75

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

ATTO 590

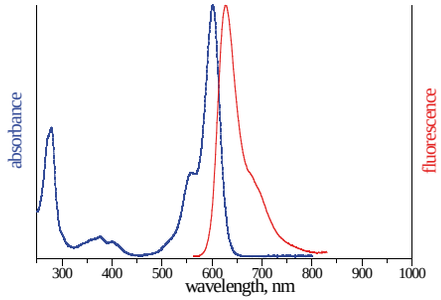
Solvent: **Water**

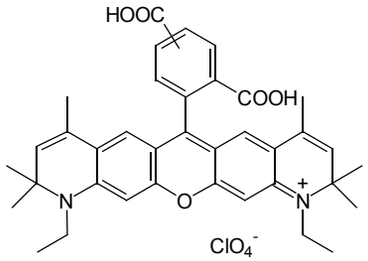
λ_{abs} = 594 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 624 nm
η_{fl} = 80 %
τ_{fl} = 3.7 ns

CF_{260} = 0.42
CF_{280} = 0.44

Features:

- High fluorescence yield
- High thermal and photostability
- Good solubility in polar solvents
- New dye related to rhodamines, perchlorate salt
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	691.2	591	1 mg 5 mg	AD 590-21 AD 590-25
NHS-ester	788.3	688	1 mg 5 mg	AD 590-31 AD 590-35
maleimide	813.3	713	1 mg 5 mg	AD 590-41 AD 590-45
biotin derivative	1001.6	901	1 mg 5 mg	AD 590-71 AD 590-75

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

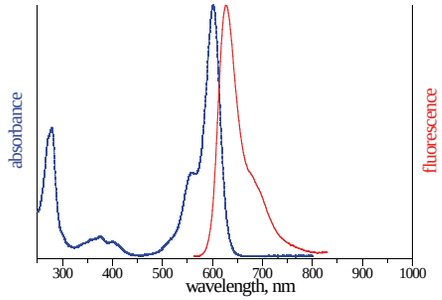
ATTO 594

Solvent: **Water**

λ_{abs}	=	601 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	627 nm			
η_{fl}	=	85 %	CF_{260}	=	0.26
τ_{fl}	=	3.5 ns	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	Unit	Order Code
with free COOH	1137	806	1 mg 5 mg	AD 594-21 AD 594-25
NHS-ester	1389	903	1 mg 5 mg	AD 594-31 AD 594-35
maleimide	1358	928	1 mg 5 mg	AD 594-41 AD 594-45
biotin derivative	1456	1116	1 mg 5 mg	AD 594-71 AD 594-75

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

Fluorescent Labels

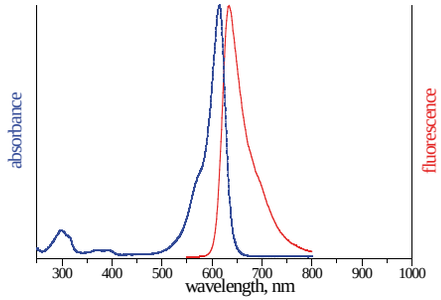
ATTO 610

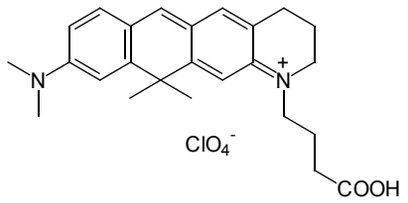
Solvent: **Water**

λ_{abs}	=	615 nm			
ε_{max}	=	1.5 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	634 nm			
η_{fl}	=	70 %	CF_{260}	=	0.02
τ_{fl}	=	3.3 ns	CF_{280}	=	0.05

Features:

- High fluorescence yield
- High photostability
- Moderately hydrophilic
- Good solubility in all polar solvents
- Stable at pH 2 - 8
- Cationic dye belonging to new class of carbopyronins, perchlorate salt





Modification	MW, g/mol	M⁺, g/mol	Unit	Order Code
with free COOH	491.0	391	1 mg 5 mg	AD 610-21 AD 610-25
NHS-ester	588.1	488	1 mg 5 mg	AD 610-31 AD 610-35
maleimide	613.1	513	1 mg 5 mg	AD 610-41 AD 610-45
biotin derivative	801.4	701	1 mg 5 mg	AD 610-71 AD 610-75

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

ATTO 611X

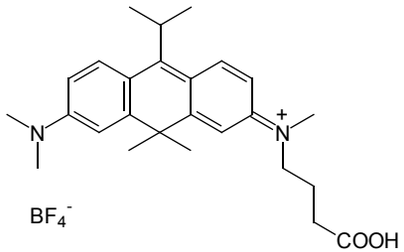
Solvent: **Water**

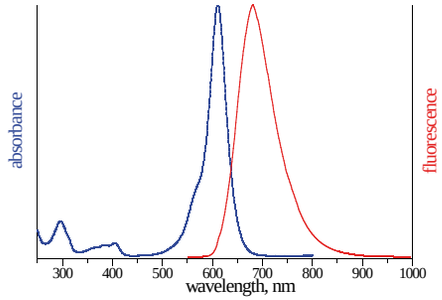
λ_{abs} = 611 nm
ε_{max} = 1.0 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 681 nm
η_{fl} = 35 %
τ_{fl} = 2.5 ns

CF_{260} = 0.05
CF_{280} = 0.07

Features:

- Fluorescence yield unusually high in this wavelength region
- Large Stokes-shift
- High photostability
- Good solubility in polar solvents
- At pH > 5 reversible formation of colorless pseudobase
- Cationic dye, tetrafluoroborate salt





Modification	MW, g/mol	M+, g/mol	Unit	Order Code
with free COOH	494.4	407	1 mg 5 mg	AD 611X-21 AD 611X-25
NHS-ester	591.5	504	1 mg 5 mg	AD 611X-31 AD 611X-35
maleimide	616.5	529	1 mg 5 mg	AD 611X-41 AD 611X-45
biotin derivative	804.8	717	1 mg 5 mg	AD 611X-71 AD 611X-75

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

ATTO 620

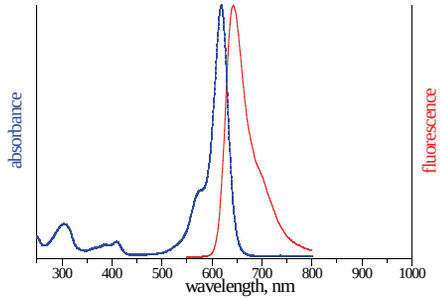
Solvent: **Water**

λ_{abs} = 619 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 643 nm
η_{fl} = 50 %
τ_{fl} = 2.9 ns

CF_{260} = 0.05
CF_{280} = 0.07

Features:

- High fluorescence yield
- High thermal and photostability
- Moderately hydrophilic
- Good solubility in all polar solvents
- Stable at pH 4 - 11
- Cationic dye, perchlorate salt



Modification	MW, g/mol	M+, g/mol	Unit	Order Code
with free COOH	612.1	512	1 mg 5 mg	AD 620-21 AD 620-25
NHS-ester	709.2	609	1 mg 5 mg	AD 620-31 AD 620-35
maleimide	734.3	634	1 mg 5 mg	AD 620-41 AD 620-45
biotin derivative	922.6	822	1 mg 5 mg	AD 620-71 AD 620-75

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

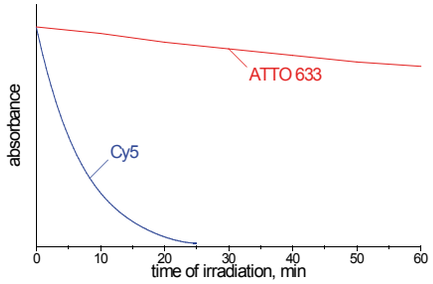
ATTO 633

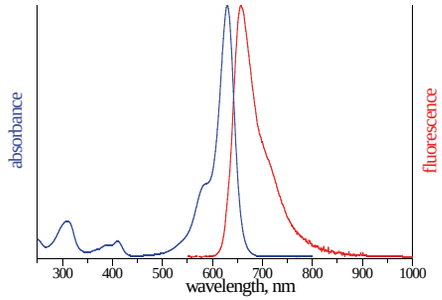
Solvent: **Water**

λ_{abs}	=	629 nm			
ε_{max}	=	1.3 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	657 nm			
η_{fl}	=	64 %	CF_{260}	=	0.05
τ_{fl}	=	3.2 ns	CF_{280}	=	0.06

Features:

- High fluorescence yield
- High thermal and photostability
- Moderately hydrophilic
- Good solubility in all polar solvents
- Stable at pH 4 - 11
- Cationic dye, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	652.2	552	1 mg 5 mg	AD 633-21 AD 633-25
NHS-ester	749.3	649	1 mg 5 mg	AD 633-31 AD 633-35
maleimide	774.3	674	1 mg 5 mg	AD 633-41 AD 633-45
biotin derivative	962.7	862	1 mg 5 mg	AD 633-71 AD 633-75

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

Fluorescent Labels

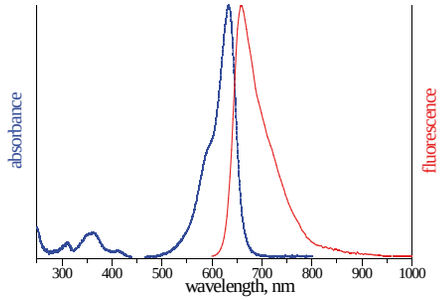
ATTO 635

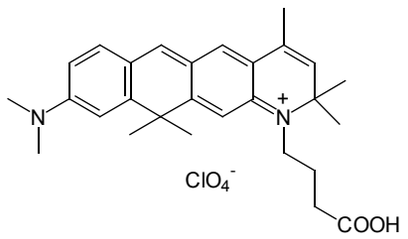
Solvent: **Water**

λ_{abs}	=	635 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	659 nm			
η_{fl}	=	25 %	CF_{260}	=	0.13
τ_{fl}	=	1.9 ns	CF_{280}	=	0.10

Features:

- High fluorescence yield
- High photostability
- Moderately hydrophilic
- Good solubility in all polar solvents
- Stable at pH 2 - 8
- Cationic dye belonging to new class of carbopyronins, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	531.1	431	1 mg 5 mg	AD 635-21 AD 635-25
NHS-ester	628.1	528	1 mg 5 mg	AD 635-31 AD 635-35
maleimide	653.2	553	1 mg 5 mg	AD 635-41 AD 635-45
biotin derivative	841.5	741	1 mg 5 mg	AD 635-71 AD 635-75

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

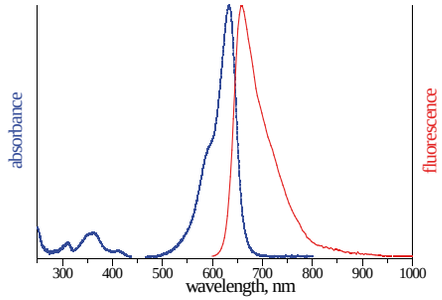
ATTO 637

Solvent: **Water**

λ_{abs}	=	635 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	659 nm			
η_{fl}	=	25 %	CF_{260}	=	0.05
τ_{fl}	=	1.9 ns	CF_{280}	=	0.02

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Good solubility in all polar solvents
- Stable at pH 2 - 8
- Zwitterionic dye



Modification	MW, g/mol	M⁺, g/mol	Unit	Order Code
with free COOH	552	511	1 mg 5 mg	AD 637-21 AD 637-25
NHS-ester	838	608	1 mg 5 mg	AD 637-31 AD 637-35
maleimide	716	633	1 mg 5 mg	AD 637-41 AD 637-45
biotin derivative	929	822	1 mg 5 mg	AD 637-71 AD 637-75

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

Fluorescent Labels

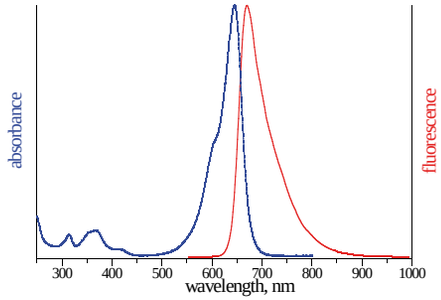
ATTO 647

Solvent: **Water**

λ_{abs}	=	645 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	669 nm			
η_{fl}	=	20 %	CF_{260}	=	0.08
τ_{fl}	=	2.3 ns	CF_{280}	=	0.04

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Good solubility in all polar solvents
- Stable at pH 2 - 8
- Zwitterionic dye



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

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

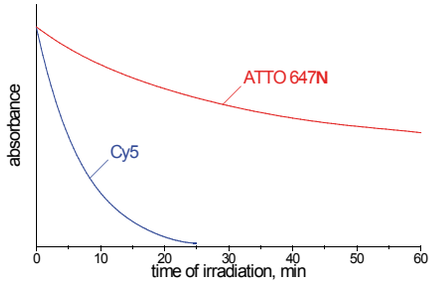
ATTO 647N

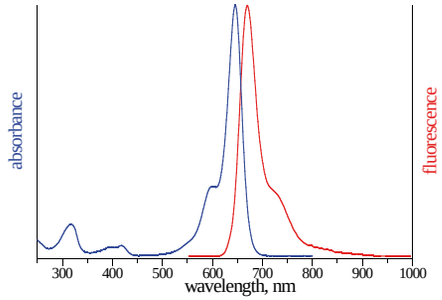
Solvent: **Water**

λ_{abs}	=	644 nm			
ε_{max}	=	1.5 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	669 nm			
η_{fl}	=	65 %	CF_{260}	=	0.06
τ_{fl}	=	3.4 ns	CF_{280}	=	0.05

Features:

- Extraordinarily high fluorescence yield at this wavelength
- High thermal and photostability
- Excellent ozone resistance
- Moderately hydrophilic
- Good solubility in polar solvents
- Stable at pH 4 - 11
- Cationic dye, perchlorate salt, mixture of two isomers





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	746.3	646	1 mg 5 mg	AD 647**N**-21 AD 647**N**-25
NHS-ester	843.4	743	1 mg 5 mg	AD 647**N**-31 AD 647**N**-35
maleimide	868.5	768	1 mg 5 mg	AD 647**N**-41 AD 647**N**-45
biotin derivative	1056.8	956	1 mg 5 mg	AD 647**N**-71 AD 647**N**-75

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

Fluorescent Labels

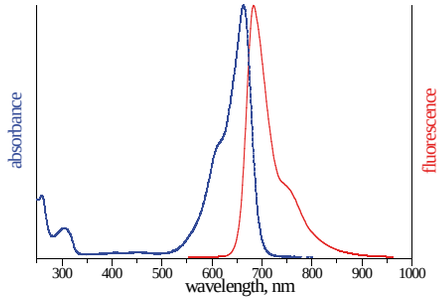
ATTO 655

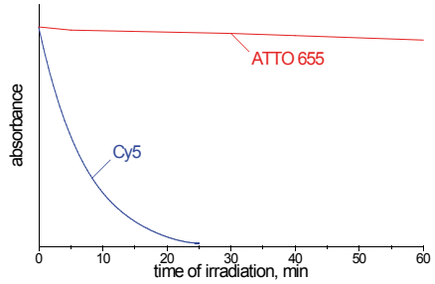
Solvent: **Water**

λ_{abs}	=	663 nm			
ε_{max}	=	1.25 x 10^5 M^{-1} cm^{-1}			
λ_{fl}	=	684 nm			
η_{fl}	=	30 %	CF_{260}	=	0.24
τ_{fl}	=	1.9 ns	CF_{280}	=	0.08

Features:

- High fluorescence yield
- Excellent thermal and photostability
- Excellent ozone resistance
- Electron transfer quenching of fluorescence by guanine, tryptophan, etc.
- Very hydrophilic
- Good solubility in all polar solvents
- Zwitterionic dye





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	634	528	1 mg 5 mg	AD 655-21 AD 655-25
NHS-ester	887	625	1 mg 5 mg	AD 655-31 AD 655-35
maleimide	812	650	1 mg 5 mg	AD 655-41 AD 655-45
biotin derivative	1204	838	1 mg 5 mg	AD 655-71 AD 655-75

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

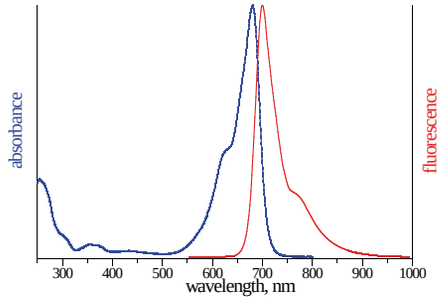
ATTO 680

Solvent: **Water**

λ_{abs}	=	680 nm			
ε_{max}	=	1.25 x 10^5 M^{-1} 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 photostability
- Electron transfer quenching of fluorescence by guanine, tryptophan, etc.
- Very hydrophilic
- Good solubility in all polar solvents
- Zwitterionic dye



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	631	526	1 mg 5 mg	AD 680-21 AD 680-25
NHS-ester	828	623	1 mg 5 mg	AD 680-31 AD 680-35
maleimide	1024	648	1 mg 5 mg	AD 680-41 AD 680-45
biotin derivative	1123	836	1 mg 5 mg	AD 680-71 AD 680-75

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

Fluorescent Labels

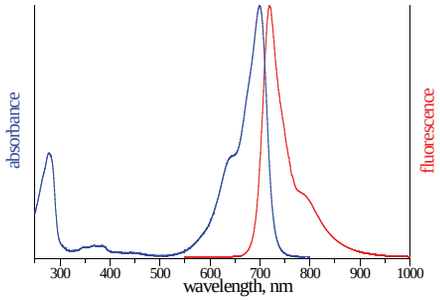
ATTO 700

Solvent: **Water**

λ_{abs}	=	700 nm			
ε_{max}	=	1.2 x 10^5 M^{-1} 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 photostability
- Electron transfer quenching of fluorescence by guanine, tryptophan, etc.
- Very hydrophilic
- Good solubility in all polar solvents
- Zwitterionic dye



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	575	566	1 mg 5 mg	AD 700-21 AD 700-25
NHS-ester	837	663	1 mg 5 mg	AD 700-31 AD 700-35
maleimide	971	688	1 mg 5 mg	AD 700-41 AD 700-45
biotin derivative	973	876	1 mg 5 mg	AD 700-71 AD 700-75

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

ATTO 725

Solvent: **Water**

λ_{abs} = 729 nm

ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}

λ_{fl} = 752 nm

η_{fl} = 10 %

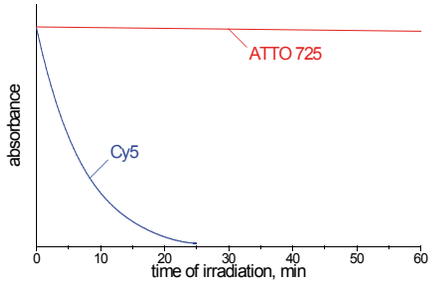
τ_{fl} = 0.5 ns

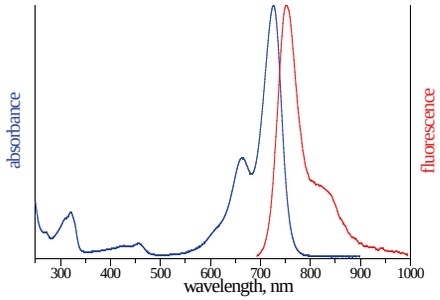
CF_{260} = 0.10

CF_{280} = 0.08

Features:

- High thermal and photostability
- Excellent photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	516.0	416	1 mg 5 mg	AD 725-21 AD 725-25
NHS-ester	613.1	513	1 mg 5 mg	AD 725-31 AD 725-35
maleimide	638.1	538	1 mg 5 mg	AD 725-41 AD 725-45
biotin derivative	826.5	726	1 mg 5 mg	AD 725-71 AD 725-75

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

Fluorescent Labels

ATTO 740

Solvent: **Water**

λ_{abs} = 740 nm

ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}

λ_{fl} = 764 nm

η_{fl} = 10 %

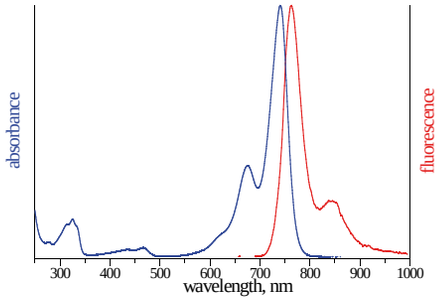
τ_{fl} = 0.6 ns

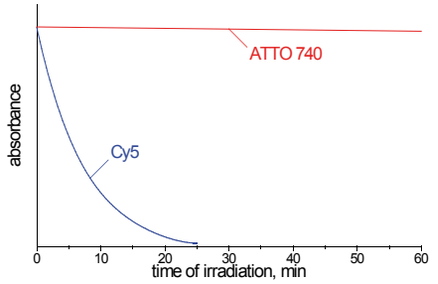
CF_{260} = 0.11

CF_{280} = 0.10

Features:

- High thermal and photostability
- Excellent photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye, perchlorate salt





Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	568.1	468	1 mg 5 mg	AD 740-21 AD 740-25
NHS-ester	665.1	565	1 mg 5 mg	AD 740-31 AD 740-35
maleimide	690.2	590	1 mg 5 mg	AD 740-41 AD 740-45
biotin derivative	878.5	778	1 mg 5 mg	AD 740-71 AD 740-75

Other conjugates with, e.g. 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. 47-49. 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.

Fluorescence Quenchers

ATTO 540Q

Solvent: **Water**

λ_{abs} = 542 nm

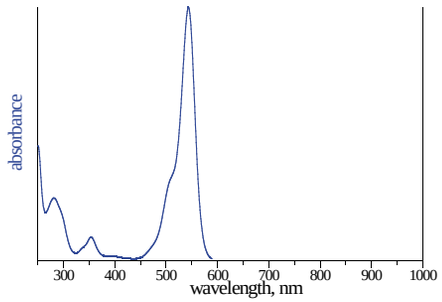
ε_{max} = 1.05 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.22

CF_{280} = 0.24

Features:

- High thermal and photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic rhodamine dye, perchlorate salt



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	659.1	559	1 mg 5 mg	AD 540Q-21 AD 540Q-25
NHS-ester	756.2	656	1 mg 5 mg	AD 540Q-31 AD 540Q-35
maleimide	781.2	681	1 mg 5 mg	AD 540Q-41 AD 540Q-45
biotin derivative	969.6	869	1 mg 5 mg	AD 540Q-71 AD 540Q-75

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

ATTO 580Q

Solvent: **Water**

λ_{abs} = 586 nm

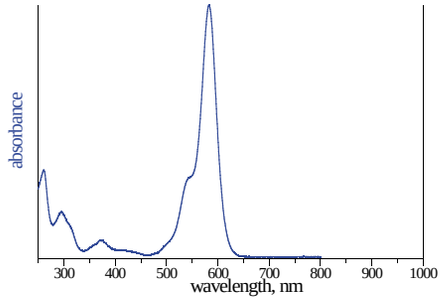
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.36

CF_{280} = 0.13

Features:

- High thermal and photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye related to rhodamines, perchlorate salt
- Supplied as mixture of three diastereomers



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	795.3	695	1 mg 5 mg	AD 580Q-21 AD 580Q-25
NHS-ester	892.4	792	1 mg 5 mg	AD 580Q-31 AD 580Q-35
maleimide	917.5	817	1 mg 5 mg	AD 580Q-41 AD 580Q-45
biotin derivative	1105.8	1005	1 mg 5 mg	AD 580Q-71 AD 580Q-75

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

Fluorescence Quenchers

ATTO 612Q

Solvent: **Water**

λ_{abs} = 615 nm

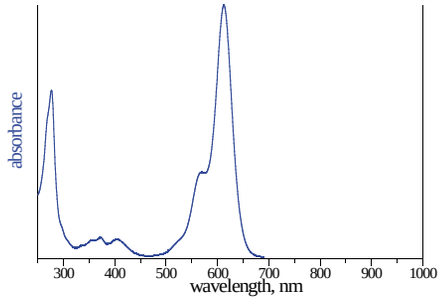
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.35

CF_{280} = 0.57

Features:

- High thermal and photostability
- Moderately hydrophilic
- Good solubility in polar solvents
- Cationic dye related to rhodamines, perchlorate salt



Modification	MW, g/mol	M^+, g/mol	Unit	Order Code
with free COOH	791.3	691	1 mg 5 mg	AD 612Q-21 AD 612Q-25
NHS-ester	888.4	788	1 mg 5 mg	AD 612Q-31 AD 612Q-35
maleimide	913.4	813	1 mg 5 mg	AD 612Q-41 AD 612Q-45
biotin derivative	1101.8	1001	1 mg 5 mg	AD 612Q-71 AD 612Q-75

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

Large Stokes-Shift Dyes

Dyes with Large Stokes-Shift

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

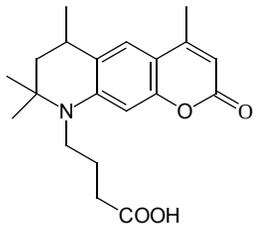
On excitation of dyes with highly *unsymmetrical* π-electron systems the dipole moment may change drastically. The ensuing strong reorientation of solvent molecules leads to an unusually large Stokes-shift, in particular in polar solvents like water and ethanol. As the non-radiative decay of the excited 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. 21).

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.

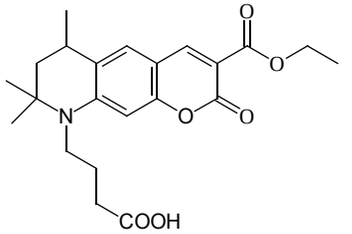
Optical Properties in Water

Label	ε_{max}, $M^{-1} cm^{-1}$	λ_{abs}, nm	λ_{fl}, nm	Stokes-Shift, nm	η_{fl}, %	τ_{fl}, ns	Page
ATTO 390	24000	390	479	89	90	3.8	22
ATTO 425	45000	436	484	48	90	3.5	23
ATTO 465	75000	453	508	55	55	2.2	24
ATTO 611X	100000	611	681	70	35	2.5	34

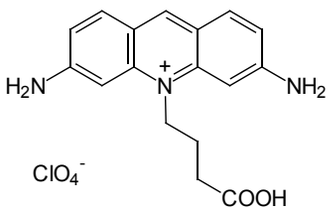
ATTO 390



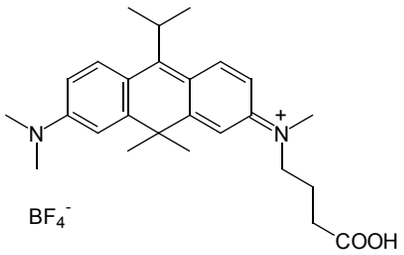
ATTO 425



ATTO 465



ATTO 611X



Customized Dyes and Services

Customized Labels and Products

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

Derivatives of ATTO-Labels

Linker

In most ATTO-labels the reactive group (NHS-ester etc.) is connected with the fluorophore by a linker consisting of a 4-atom flexible chain. For 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 amine, hydrazide (for coupling to aldehyde), 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 *streptavidin*, *phalloidin*, *sec. antibodies*, and many other proteins are prepared on request.

Special Dyes

Bichromophoric Dyes

If two fluorescent chromophores are connected by a linker, energy transfer (FRET) may occur *intra*molecularly. 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 absorbs very well in a wavelength range considerably wider than in case of a single chromophore, its fluorescence can be excited better 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.

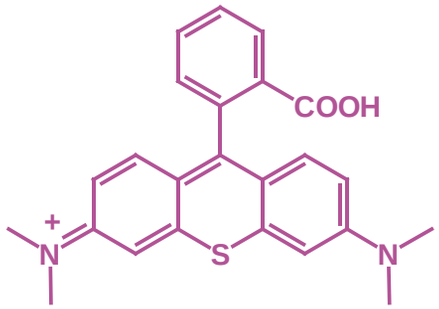
pH-Sensitive Dyes

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.

Customized Dyes and Services
ATTO-TEC

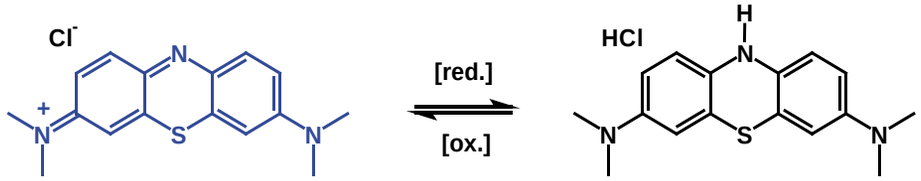
Triplet Dyes

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 excited state of the dye molecule. The occurrence of this state is frequently not desirable, as it promotes destruction (bleaching) of the dye. Nevertheless dyes with high triplet yield find application in photochemistry, photodynamic therapy etc. They are efficient sensitizers for the conversion of molecular oxygen (air) into its highly reactive form (singulet oxygen). In addition to the acridine dyes ATTO 465 (p. 24) and ATTO 495 (p. 26), both absorbing below 500 nm, we supply on customer's request triplet labels derived from *Thiorhodamine*. This dye, absorbing at 580 nm, can be provided as NHS-ester and as maleimide for coupling.



Redox Dyes

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 supplies hydrophilic derivatives of this dye activated as NHS-ester or maleimide on request.



Methylene Blue
blue

leukobase
colorless

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.

Labeling Procedures

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-hydroxysuccinimidyl(NHS)-esters. NHS-esters readily react with amine modified oligonucleotides or amino groups of proteins, i.e. the ε-amino groups of lysines or the amine terminus, forming a chemically stable amide bond between the dye and the protein or oligo. However, the amino group ought to be unprotonated to be reactive. Therefore the pH of the solution must be increased sufficiently 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 - 20 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 the amine-reactive dye in *anhydrous*, *amine-free* DMF or DMSO at 1.0 mg/ml. 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 with 1 cm path length. You may need to dilute the solution, if it turns out to be too concentrated for a correct absorbance measurement. Determine the absorbance (A_{max}) at the absorption maximum (λ_{abs}) of the dye and the absorbance (A_{280}) at 280 nm (absorption maximum of proteins). The concentration of bound dye is given by: c(dye) = A_{max} / ε_{max} × d, where ε_{max} is the extinction coefficient of the dye at the absorption maximum. The protein concentration is obtained in the same way from its absorbance at 280 nm. As all dyes show some absorption at 280 nm, the measured absorbance A_{280} must be corrected for the contribution of the dye. This is given by A_{max} × CF_{280}. The values for the correction factor CF_{280} = ε_{280} / ε_{max} are listed in the table on p.63. It follows for the absorbance of the protein itself:
$A_{prot} = A_{280} - A_{max} \times CF_{280}$. Then the concentration of protein is:
c(protein) = A_{prot} / ε_{prot} × d, where ε_{prot} is the extinction coefficient of the protein at 280 nm.

Labeling Procedures

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

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

Note: The above relation is only valid if the extinction coefficient of the free dye ε_{max} at the absorption maximum is the same as the extinction coefficient of the conjugated dye at this wavelength. Frequently this is not the case, so that the value calculated for DOL may be at fault 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 necessary with other reducing agents. We recommend to carry out the thiol modification in an inert atmosphere to prevent oxidation of the thiols, in particular if the protein has been treated with reagents such as dithiothreitol. In this case 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 ensure that all reactive dye is consumed add an excess of a low molecular weight thiol, e.g. glutathione or mercaptoethanol.

Separation of the Conjugate from Free Dye

To separate the thiol modified 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 thiol modified 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 maleimide with, e.g. mercaptoethanol.

Labeling Amine-Modified Oligonucleotides

The oligonucleotide must be functionalized with an amino group at the 5‘-end. 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 achieve a concentration of 25 µg/µl (4.2 mM for an 18-mer). This stock solution may be stored at -20 °C.

Labeling Procedures

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

Allow the amine-reactive dye solution to reach room temperature before opening the vial. Dissolve the compound in anhydrous amine-free DMF or DMSO to achieve 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.

Labeling Procedures

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 µl 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. Fore 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. In all cases 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		λ_{abs}, nm	ε_{max}, $M^{-1} cm^{-1}$	CF_{260}	CF_{280}
	NHS	Mal.				
ATTO 390	440	465	390	2.4×10^4	0.52	0.08
ATTO 425	498	523	436	4.5×10^4	0.27	0.23
ATTO 465	493	518	453	7.5×10^4	1.12	0.54
ATTO 488	981	1067	501	9.0×10^4	0.25	0.10
ATTO 495	549	574	495	8.0×10^4	0.57	0.39
ATTO 520	564	589	516	1.1×10^5	0.40	0.40
ATTO 532	1081	1063	532	1.15×10^5	0.22	0.11
ATTO 540Q	756	781	542	1.05×10^5	0.22	0.24
ATTO 550	791	816	554	1.2×10^5	0.24	0.12
ATTO 565	708	733	563	1.2×10^5	0.34	0.16
ATTO 580Q	892	917	586	1.1×10^5	0.36	0.13
ATTO 590	788	813	594	1.2×10^5	0.42	0.44
ATTO 594	1389	1358	601	1.2×10^5	0.26	0.51
ATTO 610	588	613	615	1.5×10^5	0.02	0.05
ATTO 611X	604	629	611	1.0×10^5	0.05	0.07
ATTO 612Q	888	913	615	1.15×10^5	0.35	0.57
ATTO 620	709	734	619	1.2×10^5	0.05	0.07
ATTO 633	749	774	629	1.3×10^5	0.05	0.06
ATTO 635	628	653	635	1.2×10^5	0.13	0.10
ATTO 637	838	716	635	1.2×10^5	0.05	0.02
ATTO 647	811	832	645	1.2×10^5	0.08	0.04
ATTO 647**N**	843	868	644	1.5×10^5	0.06	0.05
ATTO 655	887	812	663	1.25×10^5	0.24	0.08
ATTO 680	828	1024	680	1.25×10^5	0.30	0.17
ATTO 700	837	971	700	1.2×10^5	0.26	0.41
ATTO 725	613	638	729	1.2×10^5	0.10	0.08
ATTO 740	665	690	740	1.2×10^5	0.11	0.10

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)

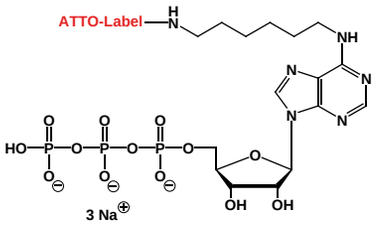
All nucleotides are available with the following ATTO-dyes. They are supplied as ready-to-use **1 mM** aqueous solutions in units of 10 to 30 µl depending on the particular nucleotide and/or label.

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

N^6-(6-Amino)hexyl-ATP

Order code:

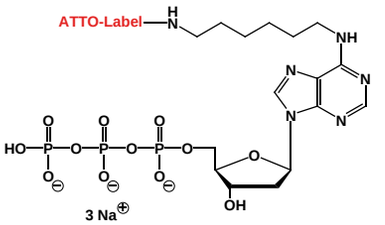
NU-805-xxx



N^6-(6-Amino)hexyl-dATP

Order code:

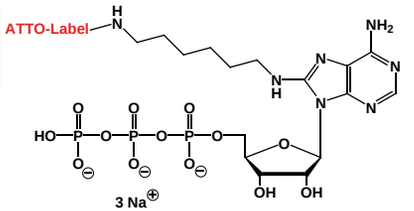
NU-835-xxx



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

Order code:

NU-807-xxx

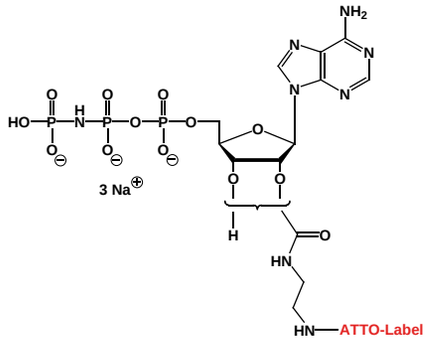


Labeled Nucleotides

EDA-AppNHp (EDA-AMPPNP)

Order code:

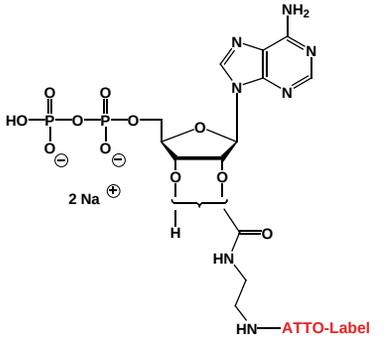
NU-810-xxx



EDA-ADP

Order code:

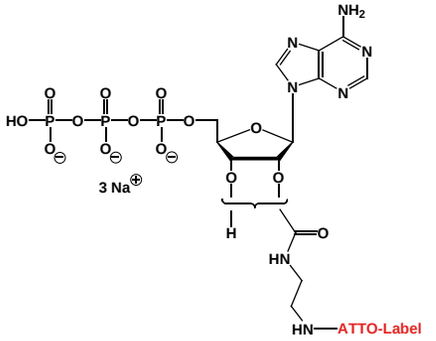
NU-802-xxx



EDA-ATP

Order code:

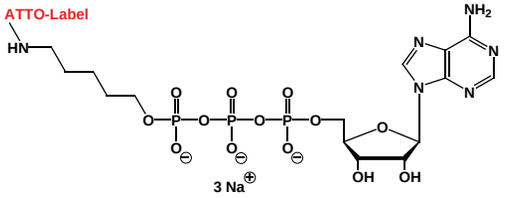
NU-808-xxx



γ-(6-Aminohexyl)-ATP

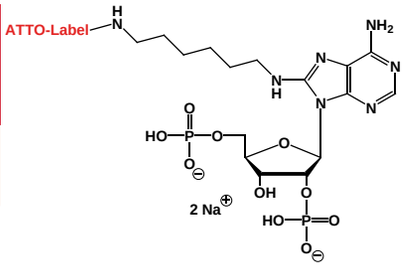
Order code:

NU-833-xxx



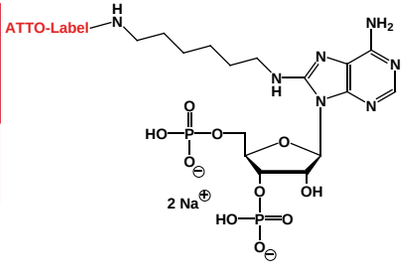
8-[(6-Amino)hexyl]-amino-adenosine-2‘,5‘-bisphosphate

Order code:
NU-812-xxx



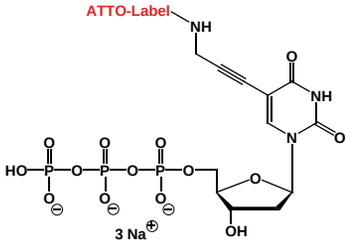
8-[(6-Amino)hexyl]-amino-adenosine-3‘,5‘-bisphosphate

Order code:
NU-811-xxx



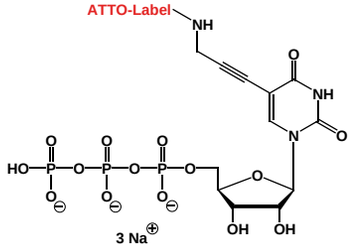
5-Propargylamino-dCTP

Order code:
NU-809-xxx



5-Propargylamino-CTP

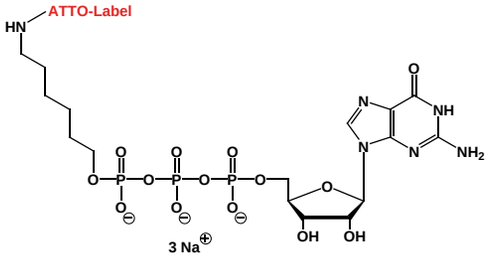
Order code:
NU-831-xxx



Labeled Nucleotides

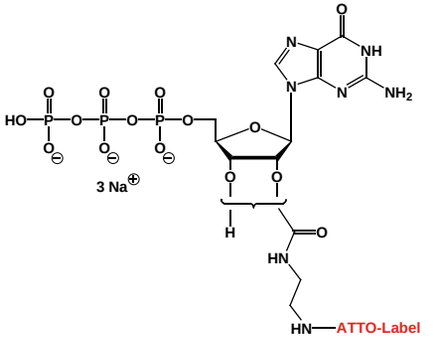
γ-(6-Aminohexyl)-GTP

Order code:
NU-834-xxx



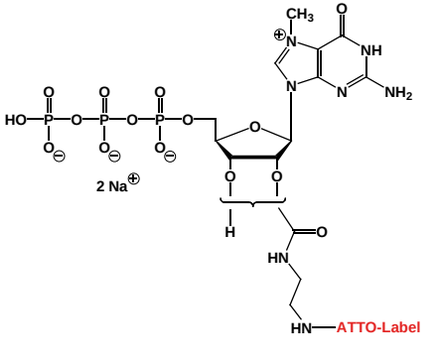
EDA-GTP

Order code:
NU-820-xxx



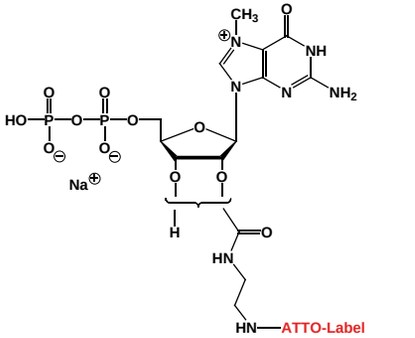
EDA-m^7-GTP

Order code:
NU-824-xxx



EDA-m^7-GDP

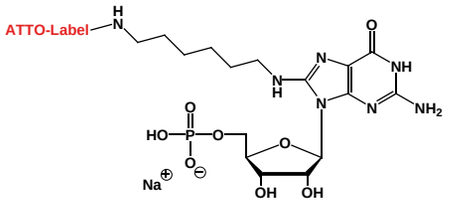
Order code:
NU-827-xxx



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

Order code:

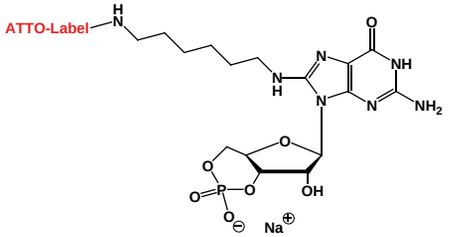
NU-829-xxx



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

Order code:

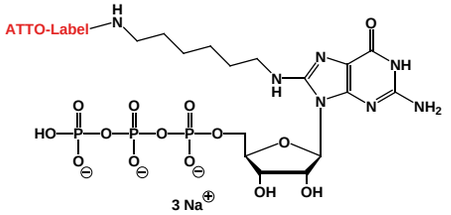
NU-832-xxx



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

Order code:

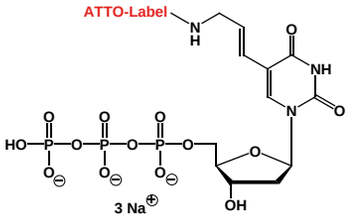
NU-830-xxx



Aminoallyl-dUTP

Order code:

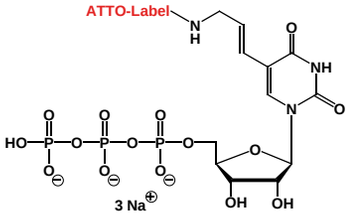
NU-803-xxx



Aminoallyl-UTP

Order code:

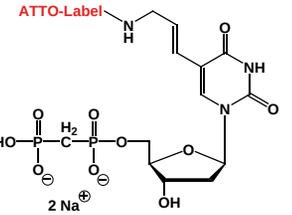
NU-821-xxx



Aminoallyl-dUpCp

Order code:

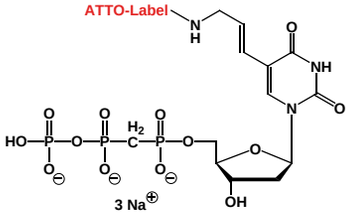
NU-825-xxx



Aminoallyl-dUpCpp

Order code:

NU-826-xxx



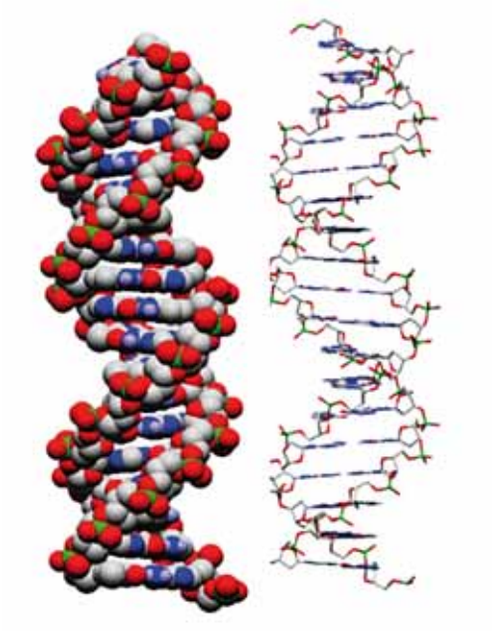
ATTO-Labels:

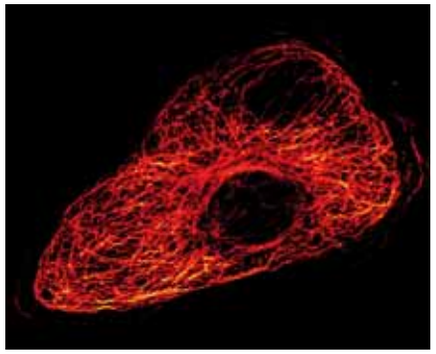
ATTO 390	ATTO 425	ATTO 465	ATTO 488	ATTO 495
ATTO 532	ATTO 540Q	ATTO 550	ATTO 565	ATTO 580Q
ATTO 590	ATTO 594	ATTO 610	ATTO 611X	ATTO 612Q
ATTO 620	ATTO 633	ATTO 635	ATTO 637	ATTO 647**N**
ATTO 655	ATTO 680	ATTO 700	ATTO 725	ATTO 740

ATTO-TEC offers a full service for synthesis and labeling of oligonucleotides. The oligonucleotides are purified via HPLC twice. Failure sequences are removed during the first HPLC; the second HPLC is performed to remove unlabeled oligonucleotide as well as excess of dye. As a result a labeled oligonucleotide of excellent quality is obtained.
The oligonucleotide can be labeled either at the 3‘ or the 5‘ end with the following dyes.

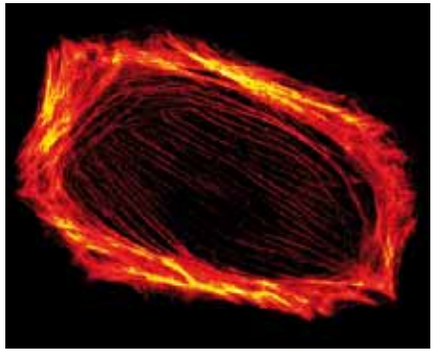
ATTO 390	ATTO 425	ATTO 465	ATTO 495	ATTO 520
ATTO 540Q	ATTO 550	ATTO 565	ATTO 580Q	ATTO 590
ATTO 594	ATTO 610	ATTO 611X	ATTO 620	ATTO 633
ATTO 635	ATTO 647**N**	ATTO 655	ATTO 680	ATTO 700
ATTO 725	ATTO 740			

All labeled oligonucleotides are supplied as aqueous solutions in PBS buffer pH 7.4.

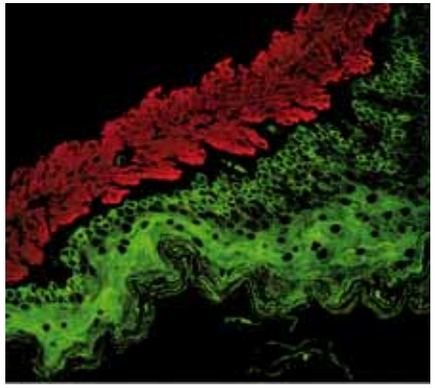




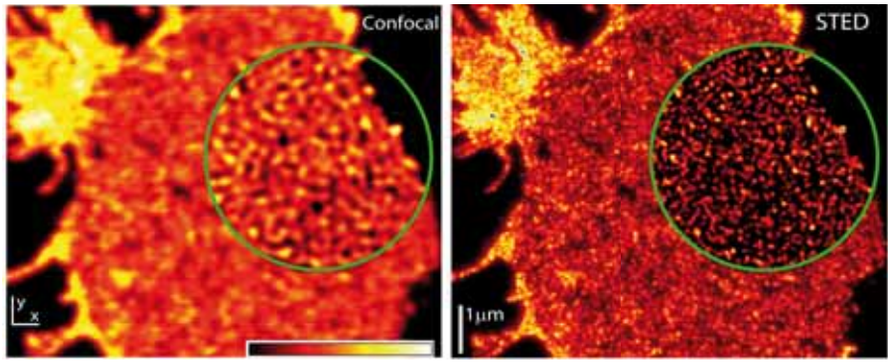
Tubulin (PtK2 - Male Rat Kangaroo Kidney Epithelial Cells)
Tubulin mouse IgG primary antibody bound to tubulin. Immunostaining with **ATTO 532** labeled sheep anti-mouse IgG secondary antibody.



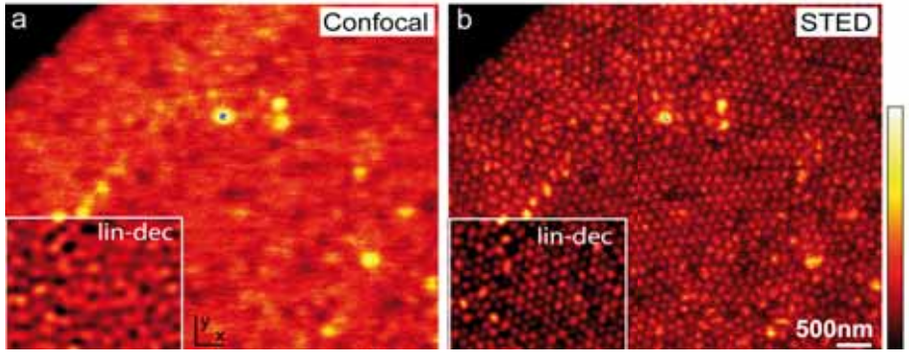
Actin (PtK2 - Male Rat Kangaroo Kidney Epithelial Cells)
ATTO 532 labeled phalloidin bound to actin.



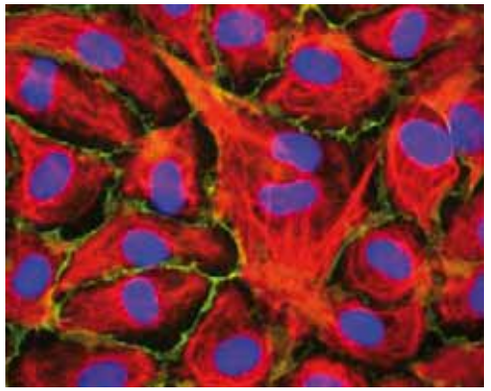
Rat stomach: Actin stained with mouse anti-smooth muscle α-actin antibody and **ATTO 488** anti-mouse IgG (green). Cytokeratin stained with polyclonal rabbit anti-cytokeratin and **ATTO 647N** anti-rabbit IgG (red).



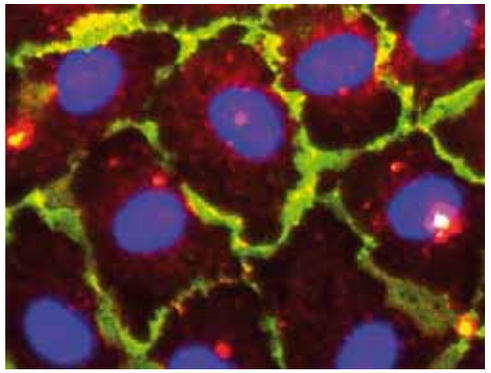
Imaging the spatial order of colloidal nanoparticles (a) Confocal image, (b) corresponding STED image. The silica nanoparticles feature a **ATTO 532** fluorescent core and a non-fluorescent shell. Only the STED image (b) reveals grain boundaries, defects and dislocations in the semi-crystalline nanoparticle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



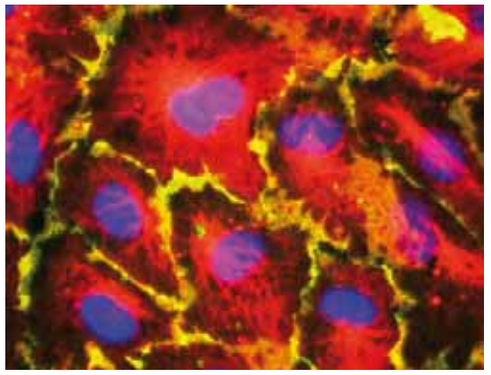
Revealing the nanopattern of the SNARE protein SNAP-25 on the plasma membrane of a mammalian cell. Confocal versus STED image of the antibody-tagged proteins. The secondary antibody was labeled with **ATTO 532-NHS**. The encircled areas show linearly deconvolved data. STED microscopy provides a substantial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



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 λ = 260 nm
ε_{280}	molar extinction coefficient at λ = 280 nm
CF_{260}	$CF_{260} = \varepsilon_{260}/\varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-DNA conjugates.
CF_{280}	$CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-protein conjugates.
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	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)
DOL	degree of labeling
HUVEC	human umbilical vein endothelial cells
DAPI	4‘,6-diamidino-2-phenylindole
FITC	fluoresceinisothiocyanate
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

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