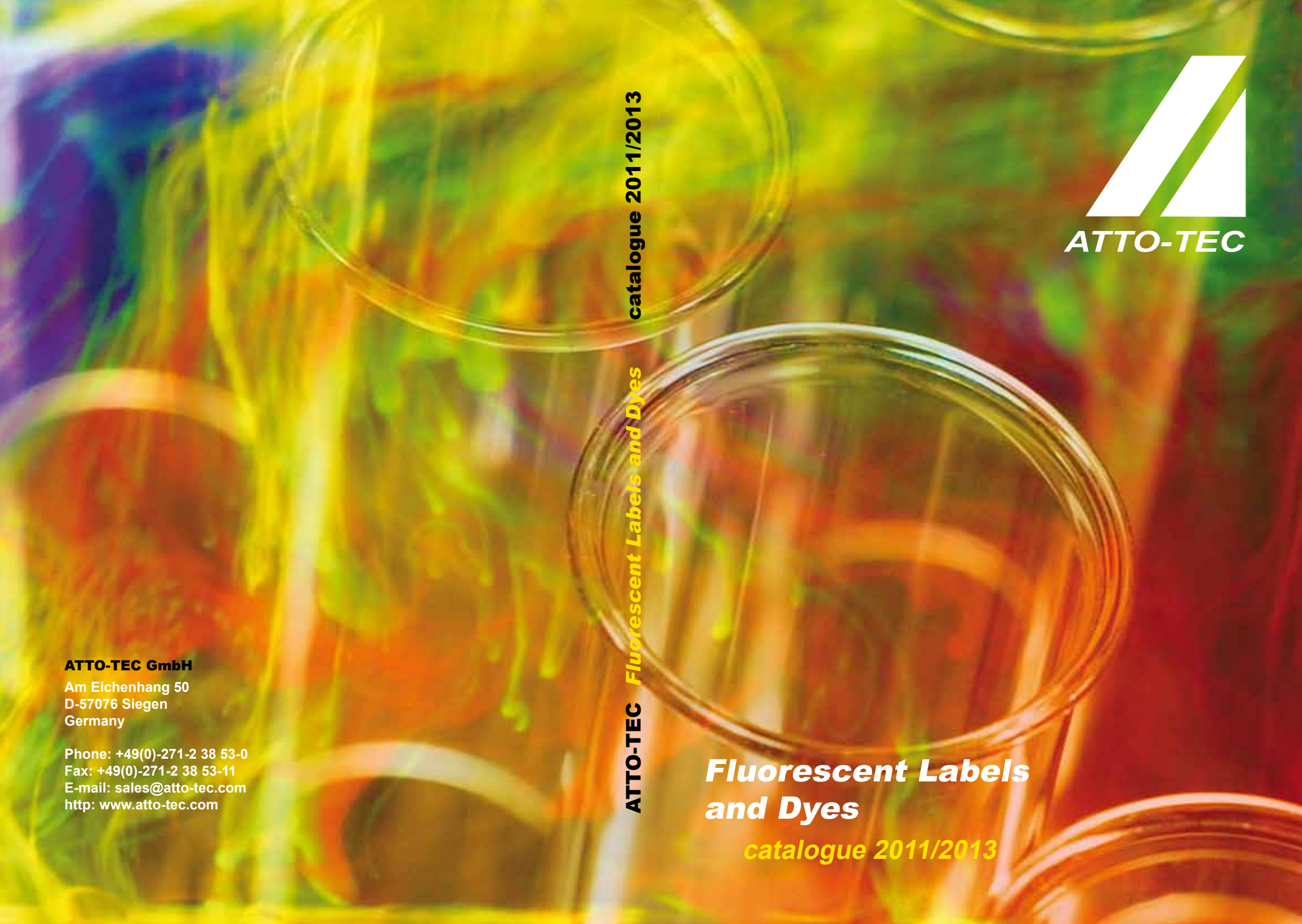

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ATTO-TEC *Fluorescent Labels and Dyes* catalogue 2011/2013

ATTO-TEC

Fluorescent Labels and Dyes

catalogue 2011/2013

Cover:

Crystals of different fluorescent ATTO dyes gradually dissolving in ethanol.

Contents

ATTO-TEC GmbH
Fluorescence - What else?

Since the start-up in 1999 **ATTO-TEC** is committed to being a first-class provider of novel fluorescent labels for bioanalytical and biomedical research. More than 40 years of academic know-how were condensed into the company in order to develop innovative solutions for the life science market. Science has always been a priority at **ATTO-TEC**. Our current staff brings together long-time expertise in dye chemistry and physics. We combine highest quality standards for all our products with an individualized customer support.

This latest catalogue 2011/2013 presents an overview of our well known ATTO-dyes, now in use world-wide. It includes many new labels and modifications. The catalogue is also meant to be a helpful introduction to the fascinating phenomenon of fluorescence. Its content is also available and continuously updated on our website (www.atto-tec.com).

We welcome any comments and suggestions in order to further improve our service and quality.

Your ATTO-TEC Team

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

ATTO-TEC

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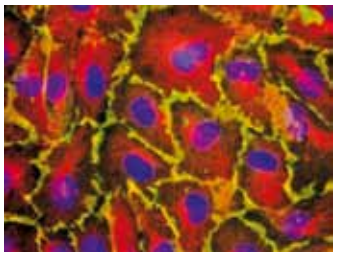

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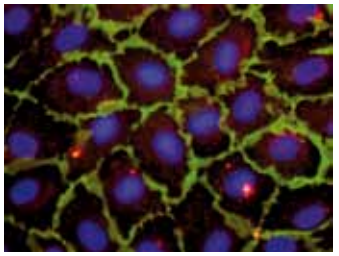

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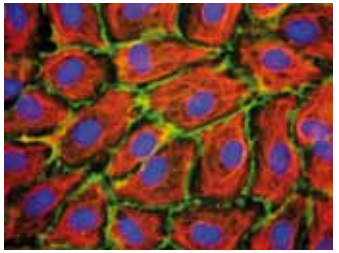


Fluorescence

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 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. 52) has a high extinction coefficient at 635 nm, a wavelength close to the maximum of the absorption curve, as well as an excellent quantum yield of fluorescence (η_{fl} = 65 %). 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 spectrum, which is always independent of excitation wavelength, 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	Suitable 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 Rho12, ATTO Rho101, ATTO 590, ATTO Rho13, ATTO 594, ATTO 610, ATTO Rho14
Argon-Ion laser	488 nm	ATTO 488, ATTO 514, ATTO 520
	514 nm	ATTO 514, ATTO 520, ATTO 532, ATTO 550
Nd:YAG laser, frequency doubled	532 nm	ATTO 532, ATTO Rho6G, ATTO 550, ATTO 565, ATTO Rho11, ATTO Rho12
He-Ne laser	633 nm	ATTO 633, ATTO 647, ATTO 647**N**, ATTO 655
Krypton-Ion laser	647 nm	ATTO 647, ATTO 647**N**, ATTO 655, ATTO Oxa12, ATTO 665, ATTO 680
	676 nm	ATTO 680, ATTO 700, ATTO 725, ATTO 740
Diode laser	635 nm	ATTO 633, ATTO 647, ATTO 647**N**, ATTO 655

Introduction

Förster 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 (donor) to the other (acceptor). The rate of energy transfer k_{ET} is according to Förster theory:

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

N_A Avogadro constant
n refractive index of solvent
τ_0 radiative decay time of donor
r distance between donor and acceptor molecule
F(λ) fluorescence spectrum of donor, normalized according to

$$\int_0^\infty F(\lambda)\, d\lambda = 1$$

ε(λ) molar decadic extinction coefficient of acceptor
κ^2 orientation factor: $\kappa^2 = (\cos\varphi_{DA} - 3\cos\varphi_D \cos\varphi_A)^2$

φ_{DA} angle between transition dipoles of donor and acceptor
φ_D angle between donor transition dipole and line connecting the dipoles
φ_A angle between acceptor transition dipole and line connecting the dipoles

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

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

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

A table of Förster-radii for ATTO-dyes is presented on p. 12-13. These values have been calculated using the value n = 1.33 (refractive index of water) and with the assumption of statistical orientation of both donor and acceptor (orientation factor κ^2 = 2/3), a situation typically encountered in solutions of unbound dye molecules. However, in case of 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.

Introduction

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

Donor	Acceptor												
ATTO	390	425	465	488	495	514	520	532	540Q	550	565	580Q	590
390	14	41	50	58	59	59	60	56	54	53	52	47	48
425		36	46	59	59	60	61	58	56	56	55	51	51
465			37	55	54	58	61	59	59	59	59	56	57
488				50	46	58	61	64	63	63	63	60	60
495					36	45	47	49	50	50	51	49	50
514						59	64	62	61	61	60	56	57
520							57	65	66	67	67	64	64
532								57	63	68	68	67	68
550										58	63	69	70
565											61	69	72
590													63
594													
610													
620													
633													
647													
647N													
655													
680													
700													
725													
740													

Acceptor												Donor
594	610	612Q	620	633	647	647N	655	680	700	725	740	ATTO
45	44	43	41	41	39	39	40	38	35	36	36	390
49	48	47	45	45	43	43	43	41	38	36	37	425
55	55	54	51	52	51	50	50	48	46	43	42	465
57	57	55	53	53	51	51	50	48	44	41	40	488
48	48	46	44	44	44	43	43	42	39	37	36	495
54	53	52	49	49	48	47	47	45	41	39	38	514
61	60	59	56	55	54	53	53	50	46	43	41	520
66	66	64	61	61	60	59	59	57	53	50	48	532
68	69	67	67	66	65	65	64	62	58	55	53	550
71	73	70	69	69	69	68	68	65	61	58	56	565
66	73	71	71	73	73	74	73	71	69	66	63	590
62	70	68	70	73	74	75	75	74	72	69	68	594
	64	63	66	70	72	73	76	75	74	69	68	610
			58	64	68	70	70	69	68	67	65	620
				60	68	69	72	73	72	72	71	633
					51	52	58	60	61	61	60	647
						65	72	75	74	73	72	647N
							58	64	66	66	65	655
								59	65	67	66	680
									58	66	66	700
										52	56	725
											54	740

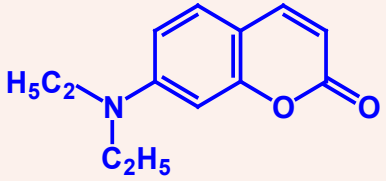
Introduction

Molecular Structure of Fluorescent Labels

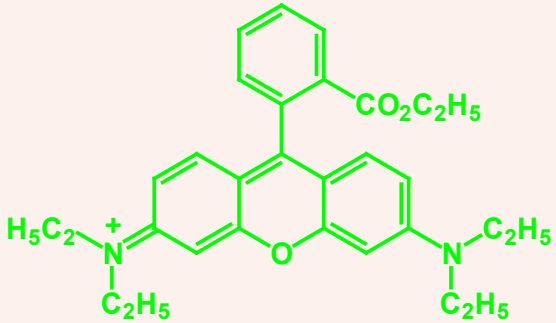
The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of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

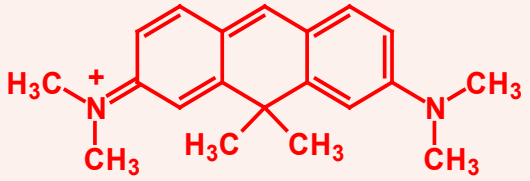
- **Coumarin**



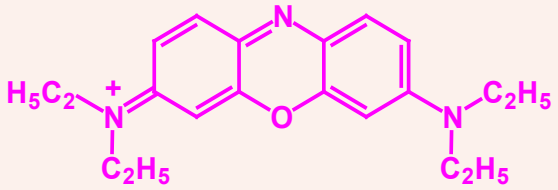
- **Rhodamine**



- **Carbopyronin**

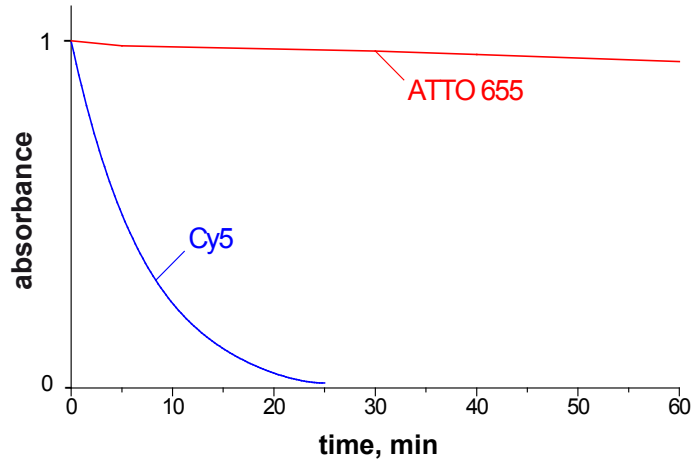


- **Oxazine**



Properties of Fluorescent Labels

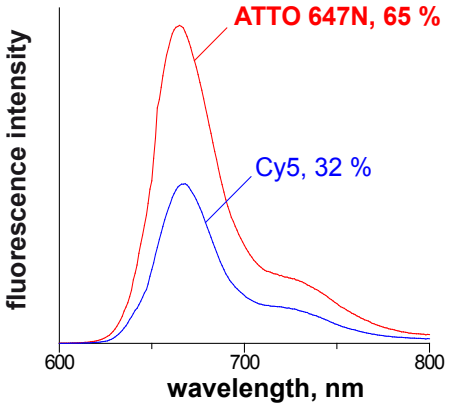
Apart from absorption and fluorescence there are many other properties that are highly relevant with respect to the suitability of dyes as labels. Most important, the dye must remain intact during irradiation. Many common labels, e.g. Fluorescein (FITC), show very low photostability. As a result sensitivity 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 Cy5™ in water. Radiation of 250 W tungsten-halogen lamp focussed into 1 cm cell. Absorbance vs. time of illumination.

Many common fluorescent labels deteriorate even without any irradiation (i.e. in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of **ozone exposure** the 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 directly exposed to the atmosphere.

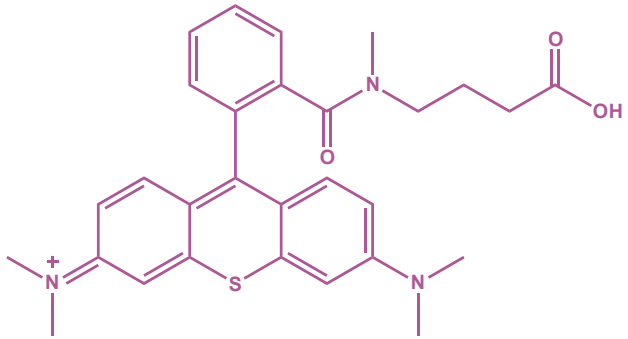
Besides reduced background, an advantage of excitation in the red spectral region is that rugged diode lasers are readily available. Diode lasers are generally less expensive and more energy-efficient than gas lasers. Furthermore a variety of sensitive detectors is available for the visible-near-IR region.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

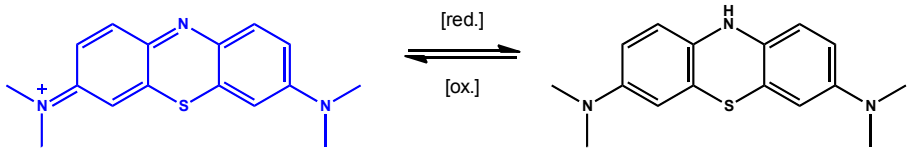
Triplet Labels

On optical excitation of a dye molecule there is always a certain probability that the molecule is converted to the *triplet* state, a relatively long-lived, non-fluorescent excited state of the dye molecule. The occurrence of this state is frequently not desirable, as it promotes destruction (bleaching) of the dye. Nevertheless dyes with high triplet yield find application in photochemistry, photodynamic therapy etc. They are efficient sensitizers for the conversion of molecular oxygen (air) into its highly reactive form (singulet oxygen). In addition to the acridine dyes **ATTO 465** (p. 30) and **ATTO 495** (p. 32), both absorbing below 500 nm, we supply **ATTO Thio12** (p. 42), 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
blue

***leuko*-form**
colorless

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. 61). 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. for 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.

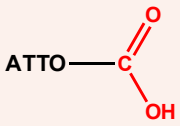
All ATTO-labels are available as NHS-esters for coupling to amino groups and as maleimides for coupling to thiol groups. In addition we offer most ATTO dyes functionalized with amine, azide (Click-Chemistry) and iodoacetamide. Dyes with other reactive substituents can be supplied on request.

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 wide-spread use of streptavidin conjugates. In this connection all ATTO-dyes are also offered as biotin conjugates. ATTO dyes conjugated to other biomolecules are available on request.

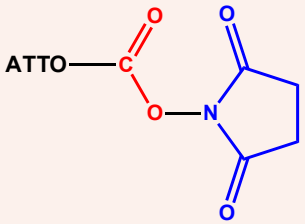
Introduction

ATTO Derivatives and Conjugates

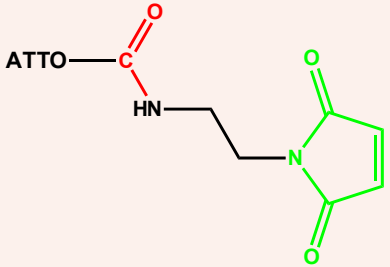
ATTO-dye with free COOH:



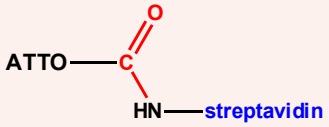
NHS-ester:



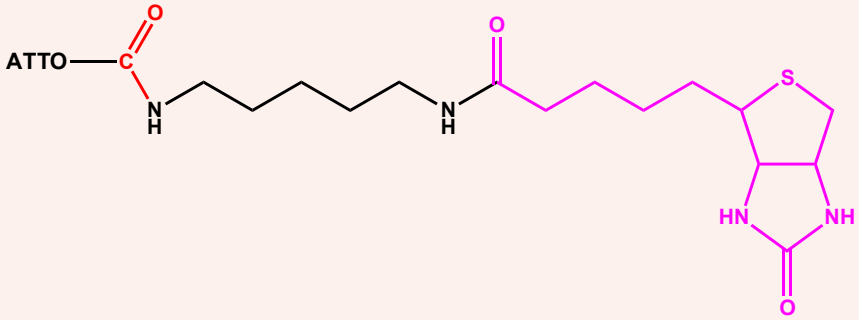
Maleimide:



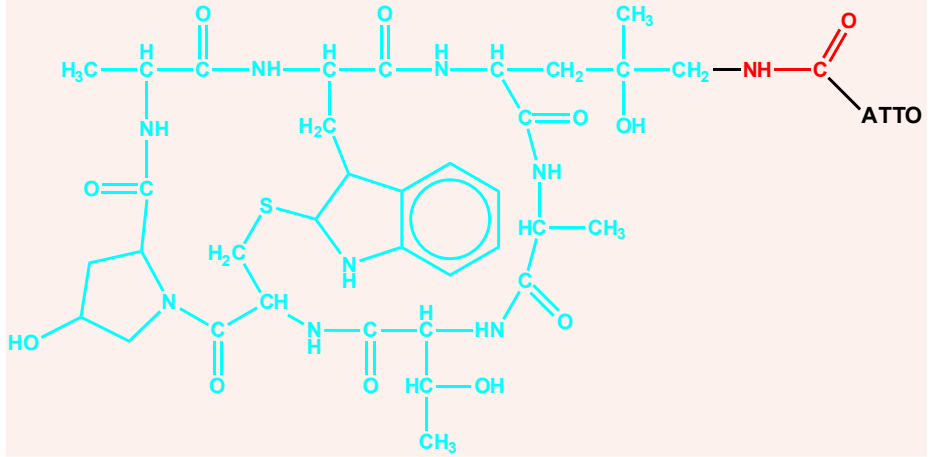
Streptavidin conjugate:



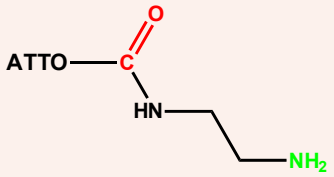
Biotin conjugate:



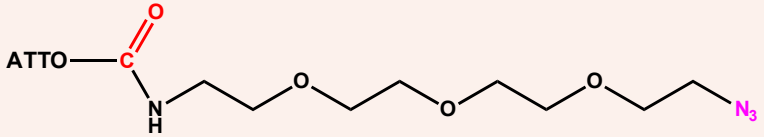
Phalloidin conjugate:



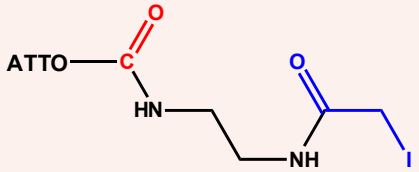
Amine:



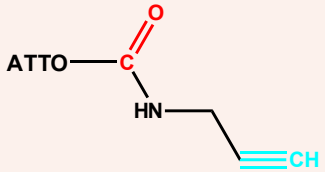
Azide:



Iodoacetamide:



Alkyne:



About this Catalogue

All spectral data given have been measured at 22 °C on aqueous solutions (PBS, pH 7.4) of the dyes with free carboxy group. When there was a tendency to aggregate, the solution was diluted sufficiently to exhibit the monomeric absorption spectrum undisturbed by dimers. Although water is the most important solvent in biochemistry, it should be borne in mind that optical data of dyes, in particular and most pronounced the fluorescence efficiency and decay time, depend on the solvent as well as on other environmental factors. With most ATTO-dyes this influence is very weak indeed. Furthermore optical properties depend on the derivative (free COOH, NHS-ester, etc.). For instance, fluorescence quantum yield and decay time 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 (*Support - Downloads - Spectra)*. - The correction factors CF_{260} and CF_{280} aid in calculating the degree of labeling (DOL), see „Labeling Procedures“ (p. 70-76).

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. The value represents the mass of the signal of maximum intensity. Counterions (An^-) - no matter, what their charge or mass - do not play any role in the labeling reactions with biomolecules.

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

Introduction

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

Increase of Molecular Mass (Δm) and Charge (Δq) on Conjugation with ATTO-Labels

ATTO-Label	Δm (NHS-ester : amine)	Δm (maleimide : thiol)	Δq
ATTO 390	325.4	465.5	0
ATTO 425	383.4	523.6	0
ATTO 465	278.4	418.5	+ 1
ATTO 488	570.6	710.7	- 1
ATTO 495	334.4	474.6	+ 1
ATTO 514	734.6	874.7	- 1
ATTO 520	349.5	489.6	+ 1
ATTO 532	626.7	766.8	- 1
ATTO Rho6G	496.6	636,7	+ 1
ATTO 550	576.8	716.9	+ 1
ATTO 565	492.6	632.7	0
ATTO Rho3B	524.7	664.8	+ 1
ATTO Rho11	548.7	688.8	+ 1
ATTO Rho12	632.9	773.0	+ 1
ATTO Thio12	484.6	624.8	+ 1
ATTO Rho101	572.7	712.9	+ 1
ATTO 590	572.7	712.8	0
ATTO Rho13	628.8	769.0	+ 1
ATTO 594	786.9	927.1	- 1
ATTO 610	373.5	513.7	+ 1
ATTO 620	494.7	634.8	+ 1
ATTO Rho14	766.6	906.8	+ 1
ATTO 633	534.7	674.9	+ 1
ATTO 647	574.8	714.9	0
ATTO 647**N**	628.9	769.0	+ 1
ATTO 655	509.6	649.8	0
ATTO Oxa12	621.9	762.0	+ 1
ATTO 665	605.7	745.9	+ 1
ATTO 680	507.6	647.8	0
ATTO 700	547.7	687.8	0
ATTO 725	398.5	538.7	+ 1
ATTO 740	450.6	590.8	+ 1
ATTO 540Q	541.6	681.8	+ 1
ATTO 580Q	677.9	818.0	+ 1
ATTO 612Q	673.8	814.0	+ 1
ATTO MB2	338.4	478.5	+ 1

ATTO-TEC
ATTO-Labels
Optical Properties

ATTO Fluorescent Labels

Label	λ_{abs}, nm	ε_{max}, M^{-1} cm^{-1}	λ_{fl}, nm	η_{fl}, %	τ_{fl}, ns	Substitute for
ATTO 390	390	24000	479	90	5.0	
ATTO 425	436	45000	484	90	3.6	
ATTO 465	453	75000	508	75	5.0	
ATTO 488	501	90000	523	80	4.1	Alexa 488*, FITC, FAM**
ATTO 495	495	80000	527	20	1.0	
ATTO 514	511	115000	533	85	3,9	Alexa 514
ATTO 520	516	110000	538	90	3.6	JOE**, TET**
ATTO 532	532	115000	553	90	3.8	Alexa 532*, HEX**
ATTO Rho6G	535	115000	560	90	4.1	HEX**
ATTO 550	554	120000	576	80	3.6	TAMRA**, Cy3***
ATTO 565	563	120000	592	90	4.0	Cy3.5***, ROX**
ATTO Rho3B	565	120000	592	50	1.5	
ATTO Rho11	571	120000	595	80	4.0	ROX**
ATTO Rho12	576	120000	601	80	4.0	
ATTO Thio12	579	110000	609	15	2.0	
ATTO Rho101	586	120000	610	80	4.2	
ATTO 590	594	120000	624	80	3.7	Alexa 594*, Texas Red*
ATTO Rho13	600	120000	625	80	3.9	
ATTO 594	601	120000	627	85	3.9	Alexa 594*
ATTO 610	615	150000	634	70	3.2	
ATTO 620	619	120000	643	50	2.9	
ATTO Rho14	625	140000	646	80	3.7	Alexa 633*
ATTO 633	629	130000	657	64	3.3	Alexa 633*
ATTO 647	645	120000	669	20	2.4	Cy5***, Alexa 647*
ATTO 647**N**	644	150000	669	65	3.5	Cy5***, Alexa 647*
ATTO 655	663	125000	684	30	1.8	Cy5***, Alexa 647*
ATTO Oxa12	663	125000	684	30	1.8	
ATTO 665	663	160000	684	60	2.9	
ATTO 680	680	125000	700	30	1.7	Cy5.5***
ATTO 700	700	120000	719	25	1.6	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	75

ATTO Triplet Labels

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 decadic extinction coefficient at the longest-wavelength absorption maximum
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	real fluorescence decay time, $\tau_{fl} = \eta_{fl} \times \tau_0$
τ_0	natural (radiative) fluorescence decay time
η_T	triplet quantum yield
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.

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

ATTO 390

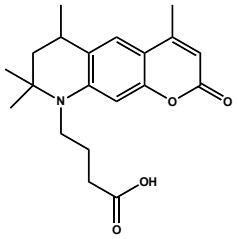
Optical properties of carboxy derivative

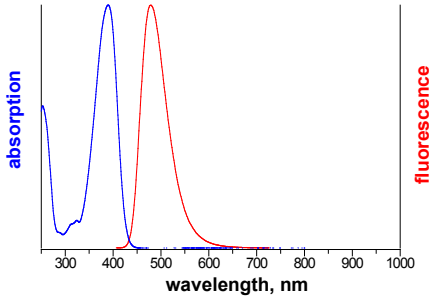
λ_{abs} = 390 nm
ε_{max} = 2.4 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 479 nm
η_{fl} = 90 %
τ_{fl} = 5.0 ns

CF_{260} = 0.52
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 1 mg	Order Code 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
streptavidin *new*			AD 390-61	AD 390-65
phalloidin	1226	1113	AD 390-81*	AD 390-82**
amine *new*	499	386	AD 390-91	AD 390-95
iodoacetamide *new*	553	554	AD 390-111	AD 390-115
alkyne *new*	494	381	AD 390-141	AD 390-145

* 10 nmol **20 nmol

Fluorescent Labels
350 nm - 500 nm

ATTO 425

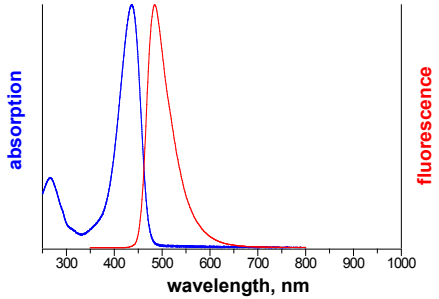
Optical properties of carboxy derivative

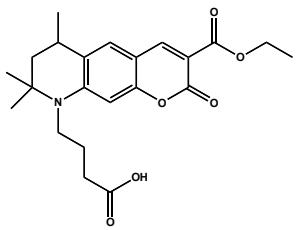
λ_{abs} = 436 nm
ε_{max} = 4.5 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 484 nm
η_{fl} = 90 %
τ_{fl} = 3.6 ns

CF_{260} = 0.27
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 1 mg	Order Code 5 mg
with free COOH	401	402	AD 425-21	AD 425-25
NHS-ester	498	499	AD 425-31	AD 425-35
maleimide	524	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

ATTO 465

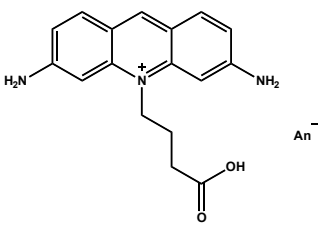
Optical properties of carboxy derivative

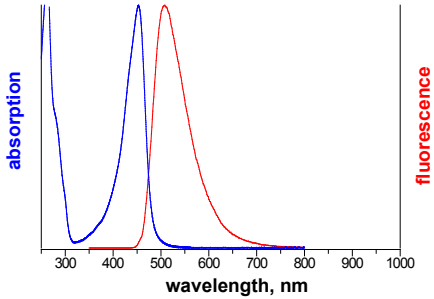
λ_{abs} = 453 nm
ε_{max} = 7.5 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 508 nm
η_{fl} = 75 %
η_T = 10 %
τ_{fl} = 5.0 ns

CF_{260} = 1.12
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 1 mg	Order Code 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 phalloidin etc. on request.

ATTO 488

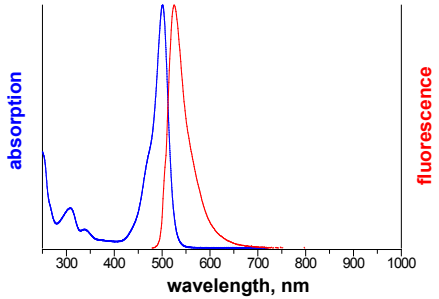
Optical properties of carboxy derivative

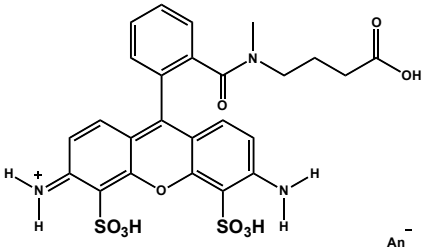
λ_{abs} = 501 nm
ε_{max} = 9.0 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 523 nm
η_{fl} = 80 %
τ_{fl} = 4.1 ns

CF_{260} = 0.25
CF_{280} = 0.10

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
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
hydrazide *new*	717	604	AD 488-121	AD 488-125
alkyne *new*	740	627	AD 488-141	AD 488-145

* 10 nmol **20 nmol

ATTO 495

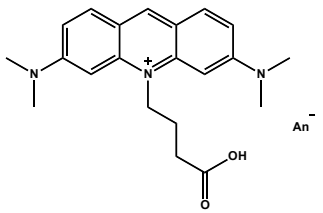
Optical properties of carboxy derivative

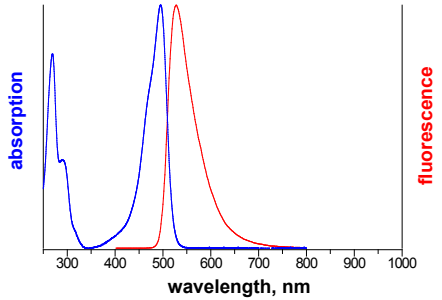
λ_{abs} = 495 nm
ε_{max} = 8.0 x 10^4 M^{-1} cm^{-1}
λ_{fl} = 527 nm
η_{fl} = 20 %
η_T = 10 %
τ_{fl} = 1.0 ns

CF_{260} = 0.57
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 1 mg	Order Code 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
biotin	762	662	AD 495-71	AD 495-75

Other conjugates with phalloidin etc. on request.

ATTO 514

New

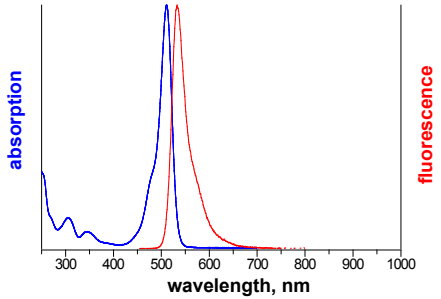
Optical properties of carboxy derivative

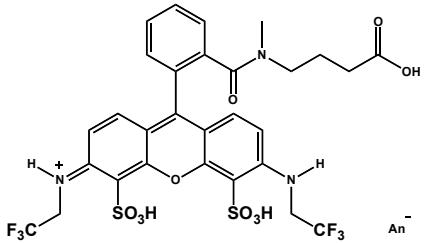
λ_{abs} = 511 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 533 nm
η_{fl} = 85 %
τ_{fl} = 3.9 ns

CF_{260} = 0.21
CF_{280} = 0.08

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 5 mg
with free COOH	867	754	AD 514-21	AD 514-25
NHS-ester	1111	851	AD 514-31	AD 514-35
maleimide	989	876	AD 514-41	AD 514-45
streptavidin			AD 514-61	AD 514-65
biotin	1177	1064	AD 514-71	AD 514-75
phalloidin	1637	1523	AD 514-81*	AD 514-82**
amine	909	796	AD 514-91	AD 514-95
azide	1067	954	AD 514-101	AD 514-105
iodoacetamide	1077	964	AD 514-111	AD 514-115
hydrazide	881	768	AD 514-121	AD 514-125
alkyne	904	791	AD 514-141	AD 514-145

* 10 nmol **20 nmol

ATTO 520

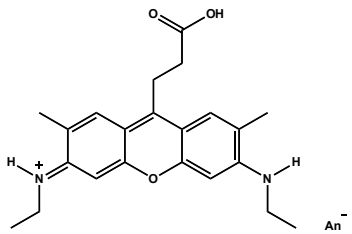
Optical properties of carboxy derivative

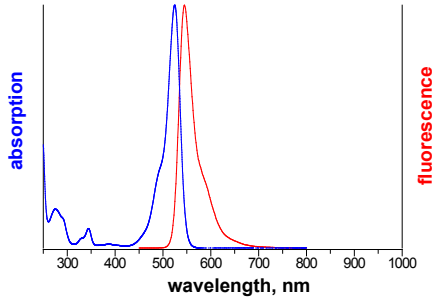
λ_{abs} = 516 nm
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 538 nm
η_{fl} = 90 %
τ_{fl} = 3.6 ns

CF_{260} = 0.13
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 1 mg	Order Code 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
phalloidin *new*	1250	1136	AD 520-81*	AD 520-82**

* 10 nmol **20 nmol

ATTO 532

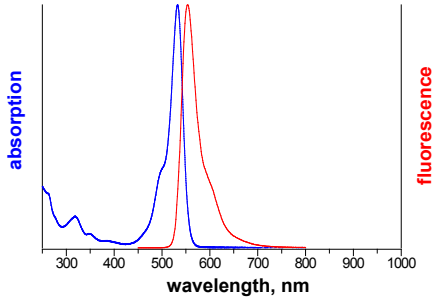
Optical properties of carboxy derivative

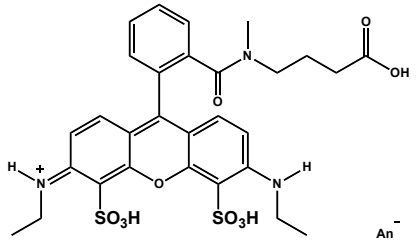
λ_{abs} = 532 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 553 nm
η_{fl} = 90 %
τ_{fl} = 3.8 ns

CF_{260} = 0.22
CF_{280} = 0.11

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
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
hydrazide *new*	773	660	AD 532-121	AD 532-125
alkyne *new*	796	683	AD 532-141	AD 532-145

* 10 nmol **20 nmol

ATTO Rho6G

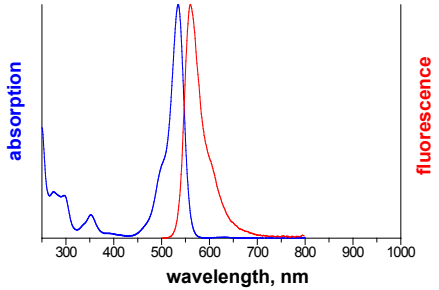
Optical properties of carboxy derivative

λ_{abs} = 535 nm
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 560 nm
η_{fl} = 94 %
τ_{fl} = 4.1 ns

CF_{260} = 0.22
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 1 mg	Order Code 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**
azide *new*	828	714	AD Rho6G-101	AD Rho6G-105
alkyne *new*	651	551	AD Rho6G-141	AD Rho6G-145

* 10 nmol **20 nmol

Other conjugates with streptavidin etc. on request.

ATTO 550

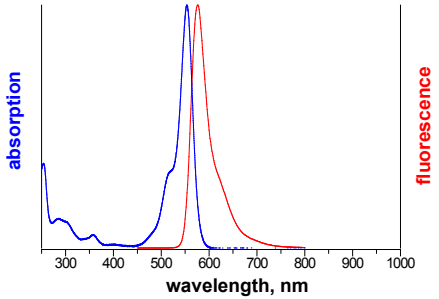
Optical properties of carboxy derivative

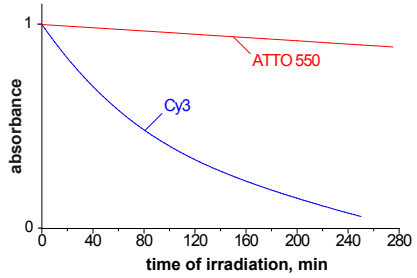
λ_{abs} = 554 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 576 nm
η_{fl} = 80 %
τ_{fl} = 3.6 ns

CF_{260} = 0.24
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 1 mg	Order Code 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
hydrazide *new*	721	608	AD 550-121	AD 550-125
alkyne *new*	731	631	AD 550-141	AD 550-145

* 10 nmol **20 nmol

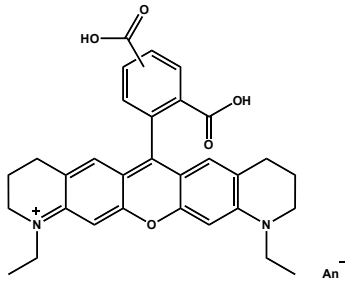
ATTO 565

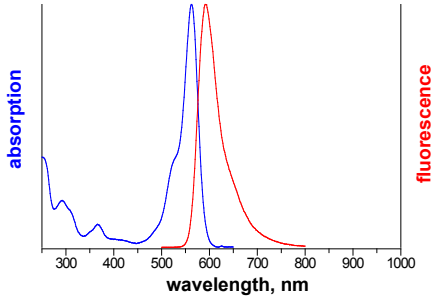
Optical properties of carboxy derivative

λ_{abs}	=	563 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	592 nm		
η_{fl}	=	90 %	CF_{260} =	0.34
τ_{fl}	=	4.0 ns	CF_{280} =	0.16

Features:

- High fluorescence yield
- High thermal and photo-stability
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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
hydrazide *new*	752	525	AD 565-121	AD 565-125
alkyne *new*	648	548	AD 565-141	AD 565-145

* 10 nmol **20 nmol

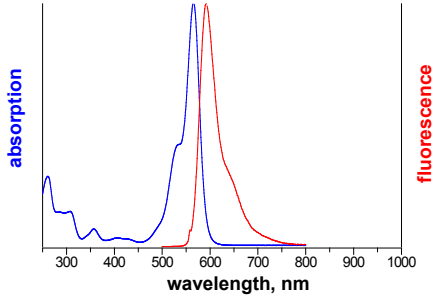
ATTO Rho3B

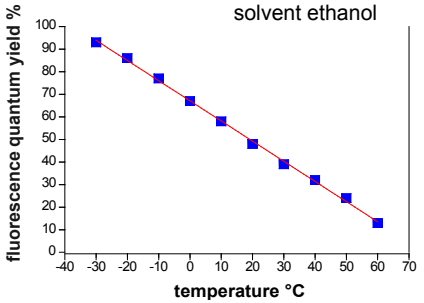
Optical properties of carboxy derivative

λ_{abs}	=	565 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	592 nm		
η_{fl}	=	50 % (in ethanol, 20°C)	CF_{260} =	0.28
τ_{fl}	=	1.5 ns	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 1 mg	Order Code 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, phalloidin etc. on request.

ATTO Rho11

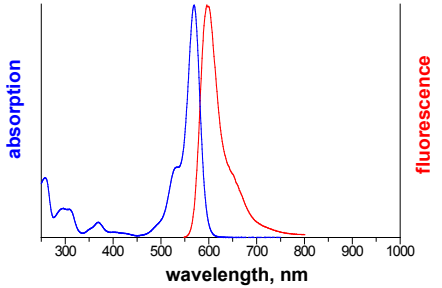
Optical properties of carboxy derivative

λ_{abs} = 571 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 595 nm
η_{fl} = 80 %
τ_{fl} = 4.0 ns

CF_{260} = 0.25
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 1 mg	Order Code 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, phalloidin etc. on request.

Fluorescent Labels
500 nm - 600 nm

ATTO Rho12

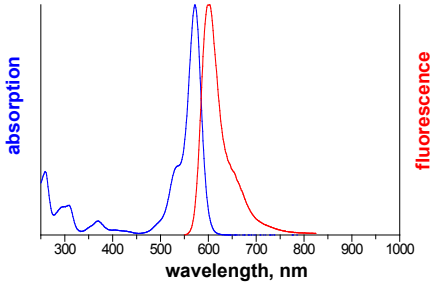
Optical properties of carboxy derivative

λ_{abs} = 576 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 601 nm
η_{fl} = 80 %
τ_{fl} = 4.0 ns

CF_{260} = 0.27
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 1 mg	Order Code 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, phalloidin etc. on request.

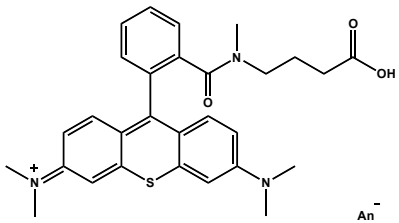
ATTO Thio12

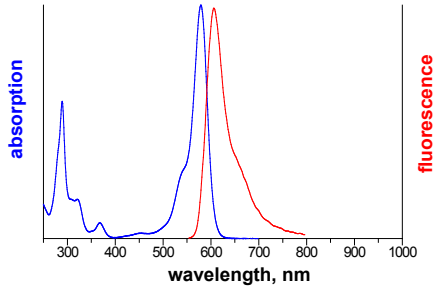
Optical properties of carboxy derivative

λ_{abs}	=	579 nm		
ε_{max}	=	1.1 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	609 nm		
η_{fl}	=	15 %	CF_{260} =	0.10
η_T	=	20 %	CF_{280} =	0.37
τ_{fl}	=	2.0 ns		

Features:

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





Modification	MW, g/mol	M⁺, g/mol	Order Code 1 mg	Order Code 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, phalloidin etc. on request.

Fluorescent Labels
500 nm - 600 nm
ATTO-TEC

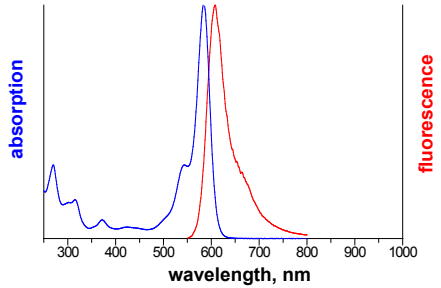
ATTO Rho101

Optical properties of carboxy derivative

λ_{abs}	=	586 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	610 nm		
η_{fl}	=	80 %	CF_{260} =	0.24
τ_{fl}	=	4.2 ns	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 1 mg	Order Code 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, phalloidin etc. on request.

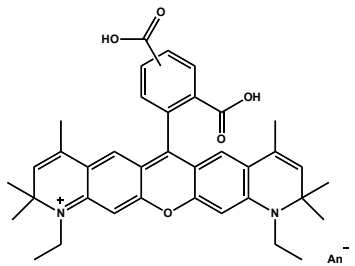
ATTO 590

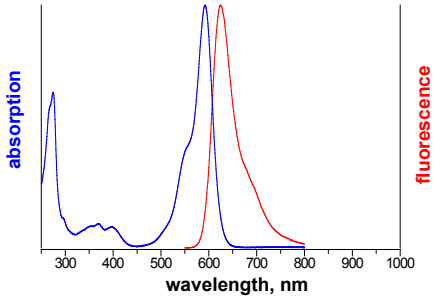
Optical properties of carboxy derivative

λ_{abs}	=	594 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	624 nm		
η_{fl}	=	80 %	CF_{260} =	0.42
τ_{fl}	=	3.7 ns	CF_{280} =	0.44

Features:

- High fluorescence yield
- High thermal and photo-stability
- Supplied as mixture of two isomers with nearly identical properties
- Single isomer on request
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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
hydrazide *new*	774	661	AD 590-121	AD 590-125
alkyne *new*	727	627	AD 590-141	AD 590-145

linker: hexamethylendiamine * 10 nmol **20 nmol

Fluorescent Labels
500 nm - 600 nm

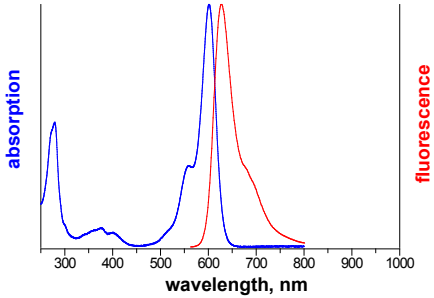
ATTO 594

Optical properties of carboxy derivative

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}	=	3.9 ns	CF_{280} =	0.51

Features:

- High fluorescence yield
- High photostability
- Very hydrophilic
- Excellent water solubility
- Very little aggregation
- Highly suitable for single-molecule applications and high-resolution microscopy
- Net charge of -1



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
phalloidin *new*	1688	1575	AD 594-81*	AD 594-95**
amine	1061	848	AD 594-91	AD 594-95
azide *new*	1119	1006	AD 594-101	AD 594-105
iodoacetamide *new*	1129	1016	AD 594-111	AD 594-115
alkyne *new*	956	843	AD 594-141	AD 594-145

* 10 nmol **20 nmol

ATTO Rho13

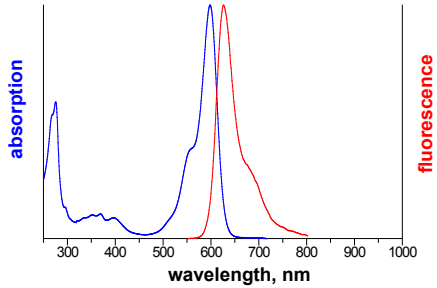
Optical properties of carboxy derivative

λ_{abs} = 600 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 625 nm
η_{fl} = 80 %
τ_{fl} = 3.9 ns

CF_{260} = 0.38
CF_{280} = 0.44

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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, phalloidin etc. on request.

ATTO 610

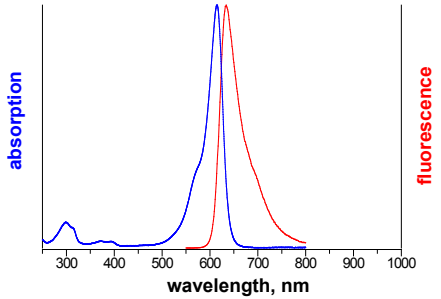
Optical properties of carboxy derivative

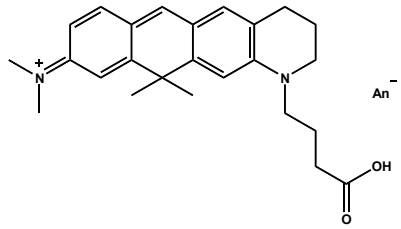
λ_{abs} = 615 nm
ε_{max} = 1.5 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 634 nm
η_{fl} = 70 %
τ_{fl} = 3.2 ns

CF_{260} = 0.02
CF_{280} = 0.05

Features:

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





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
streptavidin *new*			AD 610-61	AD 610-65
biotin	801	701	AD 610-71	AD 610-75

Other conjugates with phalloidin etc. on request.

ATTO 620

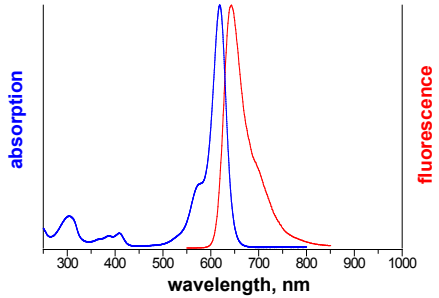
Optical properties of carboxy derivative

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:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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
streptavidin *new*			AD 620-61	AD 620-65
biotin	922	822	AD 620-71	AD 620-75

Other conjugates with streptavidin, phalloidin etc. on request.

Fluorescent Labels
600 nm - 700 nm

ATTO Rho14

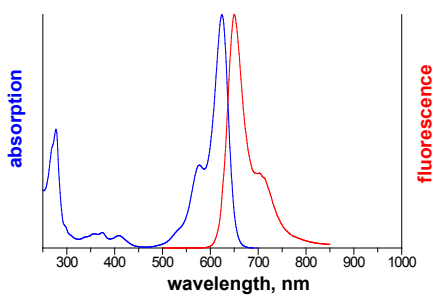
Optical properties of carboxy derivative

λ_{abs} = 625 nm
ε_{max} = 1.4 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 646 nm
η_{fl} = 80 %
τ_{fl} = 3.7 ns

CF_{260} = 0.29
CF_{280} = 0.46

Features:

- Extraordinarily high fluorescence yield
- High thermal and photo-stability
- Cationic dye



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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, phalloidin etc. on request.

ATTO 633

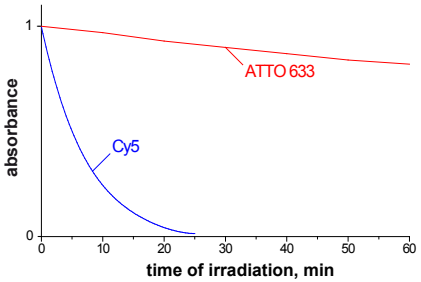
Optical properties of carboxy derivative

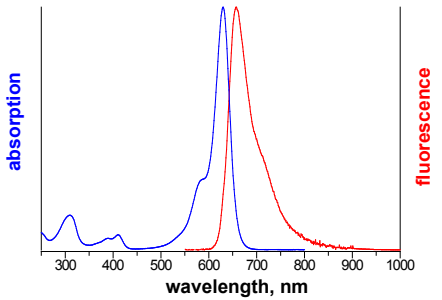
λ_{abs} = 629 nm
ε_{max} = 1.3 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 657 nm
η_{fl} = 64 %
τ_{fl} = 3.3 ns

CF_{260} = 0.05
CF_{280} = 0.06

Features:

- High fluorescence yield
- High thermal and photo-stability
- Moderately hydrophilic
- Cationic dye
- Highly suitable for single-molecule applications and high-resolution microscopy





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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
hydrazide *new*	679	566	AD 633-121	AD 633-125
alkyne *new*	689	589	AD 633-141	AD 633-145

* 10 nmol **20 nmol

ATTO 647

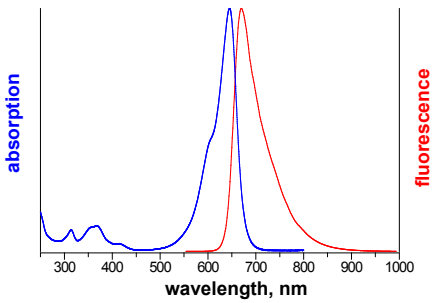
Optical properties of carboxy derivative

λ_{abs} = 645 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 669 nm
η_{fl} = 20 %
τ_{fl} = 2.4 ns

CF_{260} = 0.08
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 1 mg	Order Code 5 mg
with free COOH	592	593	AD 647-21	AD 647-25
NHS-ester	811	690	AD 647-31	AD 647-35
maleimide	828	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 phalloidin etc. on request.

ATTO 647N

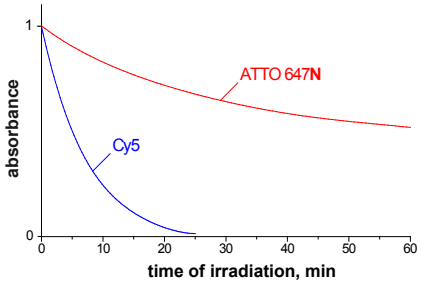
Optical properties of carboxy derivative

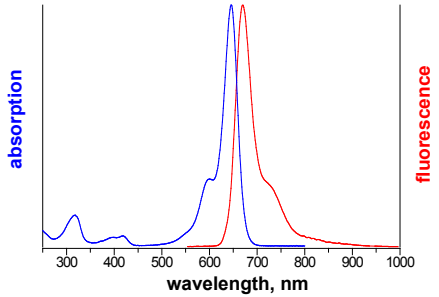
λ_{abs} = 644 nm
ε_{max} = 1.5 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 669 nm
η_{fl} = 65 %
τ_{fl} = 3.5 ns

CF_{260} = 0.06
CF_{280} = 0.05

Features:

- Extraordinarily high fluorescence yield
- High thermal and photo-stability
- Excellent ozone resistance
- Moderately hydrophilic
- Highly suitable for single-molecule applications and high-resolution microscopy
- Cationic dye, mixture of two isomers





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
with free COOH	746	646	AD 647**N**-21	AD 647**N**-25
NHS-ester	843	743	AD 647**N**-31	AD 647**N**-35
maleimide	868	768	AD 647**N**-41	AD 647**N**-45
streptavidin			AD 647**N**-61	AD 647**N**-65
biotin	1056	956	AD 647**N**-71	AD 647**N**-75
phalloidin	1528	1415	AD 647**N**-81*	AD 647**N**-82**
amine	801	688	AD 647**N**-91	AD 647**N**-95
azide	959	846	AD 647**N**-101	AD 647**N**-105
iodoacetamide	969	856	AD 647**N**-111	AD 647**N**-115
hydrazide *new*	773	660	AD 647**N**-121	AD 647**N**-125
alkyne *new*	783	683	AD 647N-141	AD 647N-145

* 10 nmol **20 nmol

ATTO 655

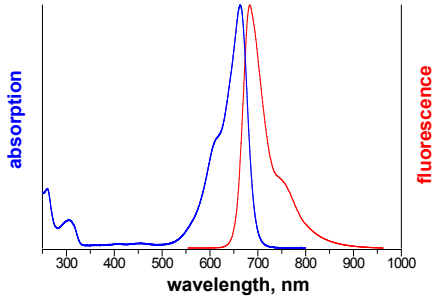
Optical properties of carboxy derivative

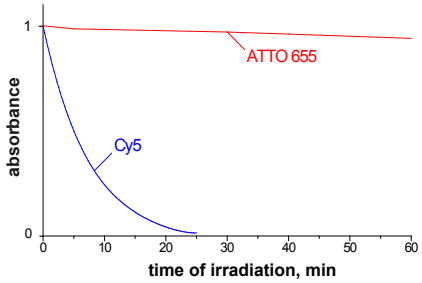
λ_{abs} = 663 nm
ε_{max} = 1.25 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 684 nm
η_{fl} = 30 %
τ_{fl} = 1.8 ns

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

Features:

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





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
alkyne *new*	841	728	AD 655-141	AD 655-145

also available with short linker: **ATTO Oxa11**. Information: info@atto-tec.com
* 10 nmol **20 nmol

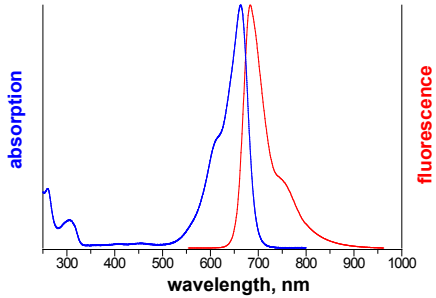
ATTO Oxa12

Optical properties of carboxy derivative

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}	=	1.8 ns	CF_{280} =	0.08

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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, phalloidin etc. on request.

Fluorescent Labels
600 nm - 700 nm

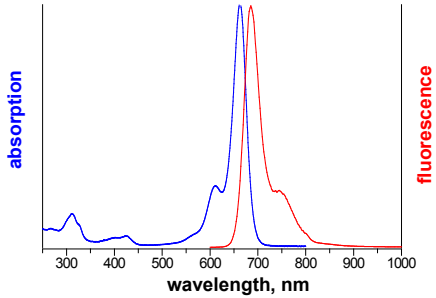
ATTO 665

Optical properties of carboxy derivative

λ_{abs}	=	663 nm		
ε_{max}	=	1.60 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	684 nm		
η_{fl}	=	60 %	CF_{260} =	0.07
τ_{fl}	=	2.9 ns	CF_{280} =	0.06

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
streptavidin *new*			AD 665-61	AD 665-65
biotin	1046	933	AD 665-71	AD 665-75
azide *new*	937	823	AD 665-101	AD 665-105

Other conjugates with streptavidin, phalloidin etc. on request.

ATTO 680

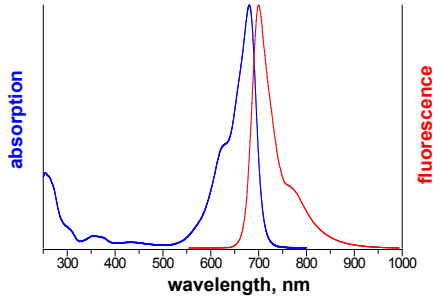
Optical properties of carboxy derivative

λ_{abs} = 680 nm
ε_{max} = 1.25 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 700 nm
η_{fl} = 30 %
τ_{fl} = 1.7 ns

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

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
phalloidin *new*	1408	1298	AD 680-81*	AD 680-82**

* 10 nmol **20 nmol

ATTO 700

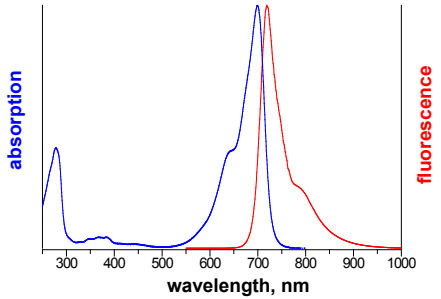
Optical properties of carboxy derivative

λ_{abs} = 700 nm
ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl} = 719 nm
η_{fl} = 25 %
τ_{fl} = 1.6 ns

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

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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 phalloidin etc. on request.

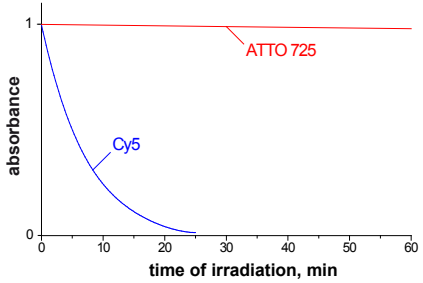
ATTO 725

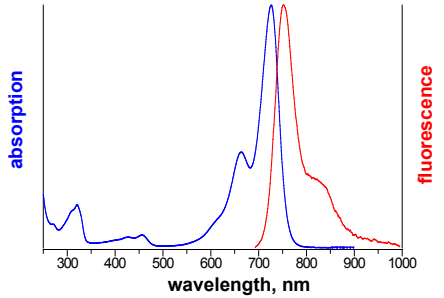
Optical properties of carboxy derivative

λ_{abs}	=	729 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	752 nm		
η_{fl}	=	10 %	CF_{260} =	0.10
τ_{fl}	=	0.5 ns	CF_{280} =	0.08

Features:

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





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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, phalloidin etc. on request.

Fluorescent Labels
700 nm - 750 nm

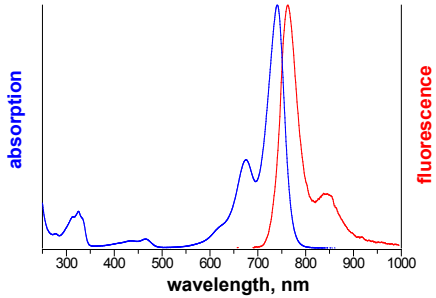
ATTO 740

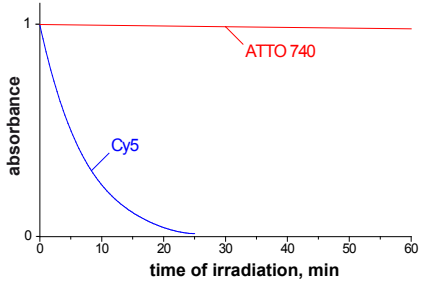
Optical properties of carboxy derivative

λ_{abs}	=	740 nm		
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}		
λ_{fl}	=	764 nm		
η_{fl}	=	10 %	CF_{260} =	0.11
τ_{fl}	=	0.6 ns	CF_{280} =	0.10

Features:

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





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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
azide *new*	782	668	AD 740-101	AD 740-105

Other conjugates with streptavidin, phalloidin etc. on request.

Redox Label

A dye, well-known in biochemical and medical research, is *Methylene Blue* (p.18-19). 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.

ATTO Redox Label

ATTO MB2

Optical properties of carboxy derivative

λ_{abs} = 658 nm

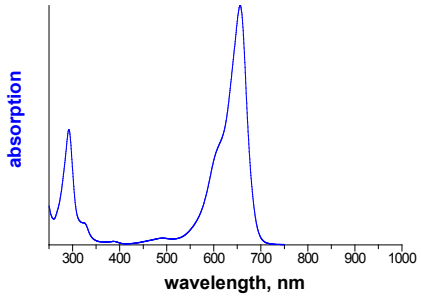
ε_{max} = 1.00 x 10^5 M^{-1} cm^{-1}

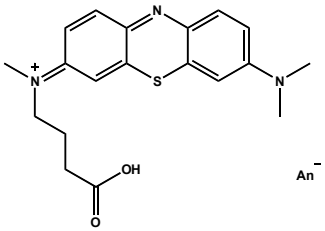
CF_{260} = 0.11

CF_{280} = 0.28

Features:

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





Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 5 mg
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, phalloidin etc. on request.

Förster resonance energy transfer (FRET) from an excited dye molecule (donor) to another nearby dye molecule (acceptor) leads to deactivation of the donor, i.e. it no longer fluoresces: Its fluorescence is *quenched*. The process of FRET depends, among other factors, on the absorption spectrum of the acceptor, as was discussed in some detail on p. 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. 63-65. The Förster-radii R_0 for combinations with fluorescent ATTO-labels as donors are presented in the table on p. 12-13.

Note:

1. The fluorescence of dyes may be quenched also by mechanisms entirely different than FRET. For example, the fluorescence of **ATTO 655**, **ATTO 680**, and **ATTO 700** is quenched very efficiently by guanosine, tryptophan and related compounds. This process is based on electron transfer and requires direct contact between excited dye molecule and quenching agent.
2. The **ATTO-TEC** quenchers **ATTO 540Q** etc. are designed to quench exclusively by the FRET mechanism. Thus, if there is no spectral overlap, no quenching takes place - in contrast to some other quenchers on the market!

Fluorescence Quenchers

ATTO 540Q

Optical properties of carboxy derivative

λ_{abs} = 542 nm

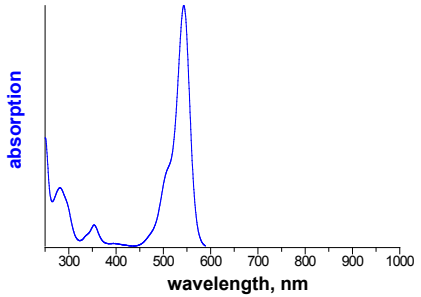
ε_{max} = 1.05 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.22

CF_{280} = 0.24

Features:

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



Modification	MW, g/mol	M^+, g/mol	Order Code 1 mg	Order Code 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
azide	873	759	AD 540Q-101	AD 540Q-105

Other conjugates with phalloidin etc. on request.

ATTO 580Q

Optical properties of carboxy derivative

λ_{abs} = 586 nm

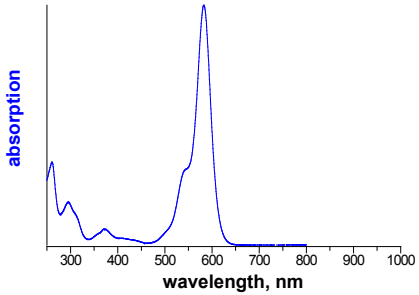
ε_{max} = 1.1 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.36

CF_{280} = 0.13

Features:

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



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 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, phalloidin etc. on request.

ATTO 612Q

Optical properties of carboxy derivative

λ_{abs} = 615 nm

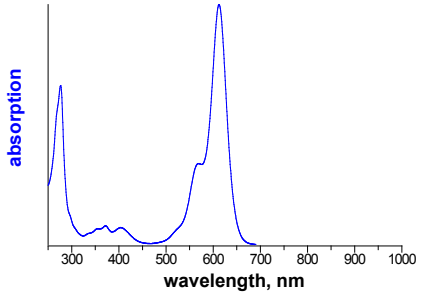
ε_{max} = 1.15 x 10^5 M^{-1} cm^{-1}

CF_{260} = 0.35

CF_{280} = 0.57

Features:

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



Modification	MW, g/mol	M+, g/mol	Order Code 1 mg	Order Code 5 mg
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 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 the Stokes-shift amounts to 20 – 30 nm.

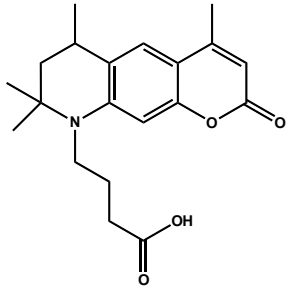
On excitation of dyes with highly *unsymmetrical* π-electron systems the dipole moment may change drastically. The ensuing strong reorientation of solvent molecules leads to an unusually large Stokes-shift, in particular in polar solvents like water and ethanol. As the non-radiative decay of the excited 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. 26).

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

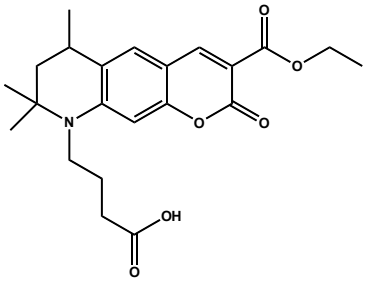
Optical Properties in Water

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	5.0	28
ATTO 425	45000	436	484	48	90	3.5	29
ATTO 465	75000	453	508	55	75	5.0	30

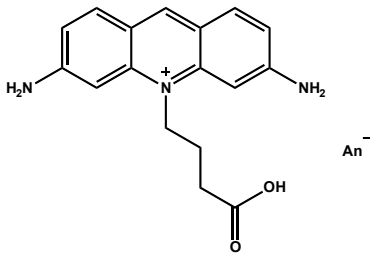
ATTO 390



ATTO 425



ATTO 465



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 and Conjugates

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-, and hydroxylamine-groups, products for “Click-Chemistry“ (azides, alkynes), AEDP (3-[(2-aminoethyl)dithio] propionic acid) as a cleavable amino acid 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.

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

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

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

Labeling Procedures

Required Materials

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

Conjugate Preparation

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

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

Conjugate Purification – Removal of Unbound Dye

- Due to an unavoidable side reaction part of the applied dye NHS-ester will hydrolyze during the labeling reaction and must be removed from the protein conjugate. We recommend using a Sephadex G-25 (or equivalent) gel filtration column (1 – 2 cm diameter and 10 – 20 cm length; for very hydrophilic dyes, e. g. ATTO 488, ATTO 532, ATTO 594, a 30 cm column is preferable) for separation of dye-protein conjugate from free dye.
- Preequilibrate the column with **Solution A**.
- Elute the dye-protein conjugate using **Solution A**.
- The first colored and fluorescent zone to elute will be the desired dye-protein conjugate. A second colored and fluorescent, but slower moving zone contains the unbound free dye (hydrolyzed NHS-ester).
- To prevent denaturation of the conjugate after elution, bovine serum albumin (BSA) or another stabilizer may be added.

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

ATTO maleimides readily react with thiol groups of proteins. The optimum acidity for thiol modification with maleimides is pH 7.0 – 7.5. At this pH the thiol (sulfhydryl) group is deprotonated to a sufficient degree and readily reacts with the dye-maleimide.

Required Materials

- **Solution A**: PBS buffer (Phosphate-Buffered Saline, pH 7.4): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4 \cdot 2\ H_2O$, and 0.24 g KH_2PO_4, in 1 liter distilled water.

Labeling Procedures

ATTO-TEC

- **Solution E**: Dissolve 1.0 mg of dye-maleimide in 50 – 200 µl of anhydrous, amine-free DMSO or DMF. Due to the high quality of ATTO maleimides such solutions are stable for a long period of time (freeze and protect from light when not in use). However, it may be difficult to avoid humidity entering a solution in continuous use. The maleimide moiety may hydrolyze and become non-reactive. Hence, we advise to freshly prepare, whenever possible, the dye-maleimide solution immediately before starting the labeling reaction.
- Gel filtration column filled with Sephadex G-25 or equivalent.

Conjugate Preparation

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

Note: If the protein contains disulfide bonds it may be desirable to reduce the disulfide before labeling. For reduction, reagents such as tris(2-carboxyethyl) phosphin (TCEP) or dithiothreitol (DTT) may be used. However, care has to be taken that any excess of these reducing agents has been removed (e. g. by dialysis) as they consume dye-maleimide themselves and in some cases even destroy the dye chromophore.

Conjugate Purification – Removal of Unbound Dye

- Part of the applied dye maleimide will hydrolyze during the labeling reaction. The unreacted maleimide and the hydrolyzed maleimide must be removed from the labeled protein. We recommend using a Sephadex G-25 (or equivalent) gel filtration column (1 – 2 cm diameter and 10 – 20 cm length; for very hydrophilic dyes, e. g. ATTO 488, ATTO 514, ATTO 532, ATTO 594, a 30 cm column is preferable) for separation of dye-protein conjugate from free dye.

- Preequilibrate the column with **Solution A**.
- Elute the dye-protein conjugate using **Solution A**.
- The first colored and fluorescent zone to elute will be the desired dye-protein conjugate. A second and maybe third colored and fluorescent, but slower moving zone contains the unreacted and/or hydrolyzed maleimide.
- To prevent denaturation of the conjugate after elution, bovine serum albumin (BSA) or another stabilizer may be added.

Storage of the Protein Conjugates

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

Determining the Degree of Labeling (DOL)

The degree of labeling (DOL, dye-to-protein ratio) obtained by the above procedures can be determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ε) × molar concentration × path length (d). Simply measure the UV-VIS spectrum of the conjugate solution as obtained after gel filtration in a quartz (UV-transparent) cell. You may need to dilute the solution, if it turns out to be too concentrated for a correct absorbance measurement. Determine the absorbance (A_{max}) at the absorption maximum (λ_{abs}) of the dye and the absorbance (A_{280}) at 280 nm (absorption maximum of proteins). The concentration of bound dye is given by: $c(dye) = A_{max} / \varepsilon_{max} \times d$, where ε_{max} is the extinction coefficient of the dye at the absorption maximum. The protein concentration is obtained in the same way from its absorbance at 280 nm. As all dyes show some absorption at 280 nm, the measured absorbance A_{280} must be corrected for the contribution of the dye. This is given by $A_{max} \times CF_{280}$. The values for the correction factor $CF_{280} = \varepsilon_{280} / \varepsilon_{max}$ are listed in the table on p.76. It follows for the absorbance of the protein itself:
$A_{prot} = A_{280} - A_{max} \times CF_{280}$. Then the concentration of protein is:
$c(protein) = A_{prot} / \varepsilon_{prot} \times d$, where ε_{prot} is the extinction coefficient of the protein at 280 nm.
It follows for the degree of labeling, i.e. the average number of dye molecules coupled to a protein molecule: DOL = c(dye) / c(protein) and with the above relations:

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

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

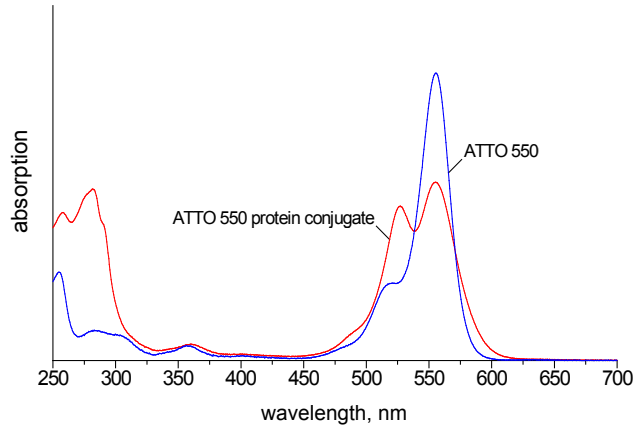


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Table: Optical properties of ATTO-labels

Dye	MW, g/mol NHS	MW, g/mol Mal	λ_{abs}, nm	ε_{max}, M^{-1} cm^{-1}	CF_{260}	CF_{280}
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 514	1111	989	511	1.15×10^5	0.21	0.08
ATTO 520	564	589	516	1.1×10^5	0.13	0.18
ATTO 532	1081	1063	532	1.15×10^5	0.22	0.11
ATTO Rho6G	711	849	535	1.15×10^5	0.22	0.19
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 Rho3B	642	764	565	1.2×10^5	0.28	0.14
ATTO Rho11	763	788	571	1.2×10^5	0.25	0.09
ATTO Rho12	847	872	576	1.2×10^5	0.27	0.09
ATTO Thio12	699	824	579	1.1×10^5	0.10	0.37
ATTO Rho101	787	812	586	1.2×10^5	0.24	0.19
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 Rho13	843	867	600	1.2×10^5	0.38	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 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 Rho14	981	1019	625	1.4×10^5	0.29	0.46
ATTO 633	749	774	629	1.3×10^5	0.05	0.06
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 Oxa12	835	874	663	1.25×10^5	0.24	0.08
ATTO 665	820	845	663	1.60×10^5	0.07	0.06
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
ATTO MB2	553	591	658	1.0×10^5	0.11	0.28

New

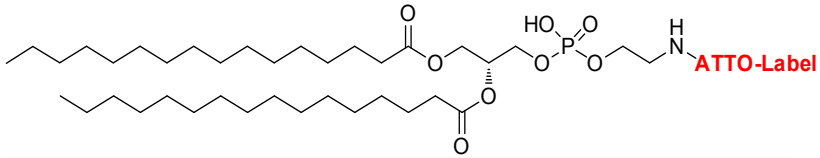
Fluorescence Labeled Membrane Probes

The investigation of biological membranes, e.g. intracellular membranes of live cells, plasma membranes etc., has become a major area of interest. As a result there is a growing demand for fluorescent lipids, in particular phospholipids to be incorporated in biological membranes. **ATTO-TEC** now offers a variety of phospholipids based on glycerol carrying one or two fatty acids (lipophilic groups) and a phosphate monoester residue (hydrophilic group).

Natural phospholipids are the predominant building blocks of biological membranes and are generally very similar in structure. However, minor differences, e.g. number and length of the fatty acid chains, degree of unsaturation of the fatty acid and nature of hydrophilic head group may result in significant variations of the physical properties and biological activity of such membranes.

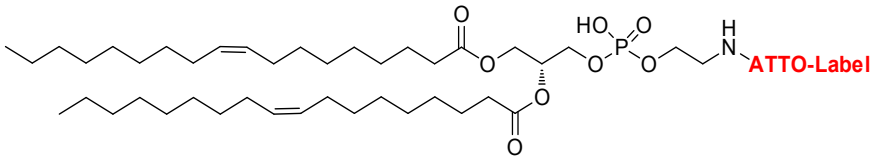
ATTO-fluorescent phospholipids are labeled at the hydrophilic head group. After incorporation of the fluorescent phospholipid the fluorophore is located at the water/lipid interface of the membrane. Currently we provide **1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine** (DPPE), **1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine** (DOPE), **1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine** (PPE), and **1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine** (DMPE) labeled with selected ATTO-dyes. We label other ATTO-dyes on request. Also for other labeled phospholipids including sphingolipids such as **sphingomyelin** please send an e-mail to info@atto-tec.com

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



ATTO-Label	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 DPPE	1263	AD 488-151	AD 488-155
ATTO 520 DPPE	1040	AD 520-151	AD 520-155
ATTO 532 DPPE	1319	AD 532-151	AD 532-155
ATTO 550 DPPE	1367	AD 550-151	AD 550-155
ATTO 590 DPPE	1380	AD 590-151	AD 590-155
ATTO 594 DPPE	1479	AD 594-151	AD 594-155
ATTO 633 DPPE	1325	AD 633-151	AD 633-155
ATTO 647**N** DPPE	1419	AD 647**N**-151	AD 647**N**-155

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



ATTO-Label	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 DOPE	1316	AD 488-161	AD 488-165
ATTO 532 DOPE	1371	AD 532-161	AD 532-165
ATTO 550 DOPE	1419	AD 550-161	AD 550-165
ATTO 590 DOPE	1415	AD 590-161	AD 590-165
ATTO 594 DOPE	1531	AD 594-161	AD 594-165
ATTO 633 DOPE	1377	AD 633-161	AD 633-165
ATTO 647**N** DOPE	1485	AD 647**N**-161	AD 647**N**-165
ATTO 655 DOPE	1366	AD 655-161	AD 655-165
ATTO 680 DOPE	1364	AD 680-161	AD 680-165

Labeled Phospholipids

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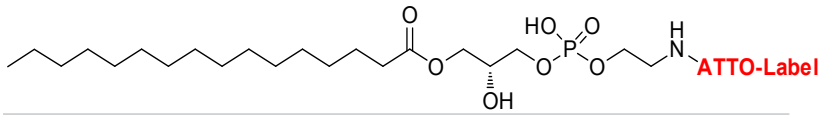
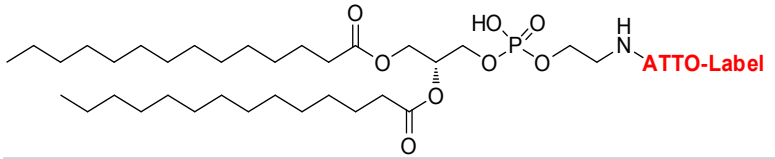
1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (PPE)



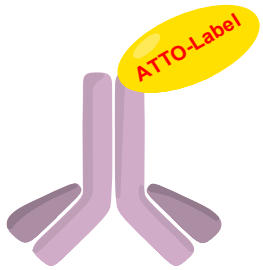
ATTO-Label	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 PPE	1024	AD 488-181	AD 488-185
ATTO 532 PPE	1080	AD 532-181	AD 532-185
ATTO 550 PPE	1127	AD 550-181	AD 550-185
ATTO 590 PPE	1123	AD 590-181	AD 590-185
ATTO 594 PPE	1240	AD 594-181	AD 594-185
ATTO 633 PPE	1085	AD 633-181	AD 633-185
ATTO 647**N** PPE	1194	AD 647**N**-181	AD 647**N**-185

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



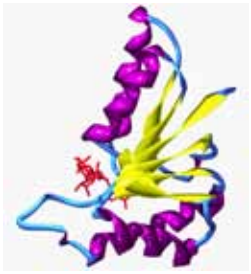
ATTO-Label	MW, g/mol	Order Code 1 mg	5 mg
ATTO 488 DMPE	1207	AD 488-191	AD 488-195
ATTO 532 DMPE	1263	AD 532-191	AD 532-195
ATTO 550 DMPE	1311	AD 550-191	AD 550-195
ATTO 590 DMPE	1307	AD 590-191	AD 590-195
ATTO 594 DMPE	1423	AD 594-191	AD 594-195
ATTO 633 DMPE	1269	AD 633-191	AD 633-195
ATTO 647**N** DMPE	1363	AD 647**N**-191	AD 647**N**-195

Fluorescence-Labeled Antibodies



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

Fluorescence-Labeled Nucleotides



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 aqueous solutions in various units and concentrations depending on the particular nucleotide and/or label.
For detailed information please visit www.jenabioscience.com.

Available ATTO-dye labeled nucleotides:

Labeled Uridine Nucleotides

- Aminoallyl-UTP (NU-821-xxx)
- Aminoallyl-dUTP (NU-803-xxx)
- 5-Propargylamino-ddUTP (NU-1619-xxx)

Labeled Cytidine Nucleotides

- 5-Propargylamino-CTP (NU-831-xxx)
- 5-Propargylamino-dCTP (NU-809-xxx)
- 5-Propargylamino-ddCTP (NU-850-xxx)

Labeled Guanosine and m^7 Guanosine Nucleotides

- 8-[(6-Amino)hexyl]-amino-GMP (NU-829-xxx)
- 8-[(6-Amino)hexyl]-amino-cGMP (NU-832-xxx)
- γ-[6-Aminohexyl]-GTP (NU-834-xxx)
- EDA-GTP (NU-820-xxx)
- 8-[(6-Amino)hexyl]-amino-GTP (NU-830-xxx)
- EDA-m^7 GDP (NU-827-xxx)
- EDA-m^7 GTP (NU-824-xxx)
- 7-Propargylamino-7-deaza-dGTP (NU-1615-xxx)
- 7-Propargylamino-7-deaza-ddGTP (NU-1618-xxx)

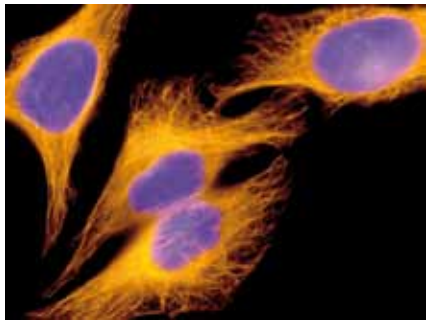
Labeled Adenosine Nucleotides

- EDA-ADP (NU-802-xxx)
- N6-(6-Amino)hexyl-ATP (NU-805-xxx)
- 8-[(6-Amino)hexyl]-amino-ATP (NU-807-xxx)
- EDA-ATP (NU-808-xxx)
- γ-[6-Aminohexyl]-ATP (NU-833-xxx)
- EDA-AppNHp (NU-810-xxx)
- 8-[(6-Amino)hexyl]-amino-adenosine-2’,5’-bisphosphate (NU-812-xxx)
- 8-[(6-Amino)hexyl]-amino-adenosine-3’,5’-bisphosphate (NU-811-xxx)
- N6-(6-Amino)hexyl-dATP (NU-835-xxx)
- 8-[(6-Amino)hexyl]-amino-cAMP (NU-851-xxx)
- 7-Propargylamino-7-deaza-dATP (NU-1611-xxx)
- 7-Propargylamino-7-deaza-ddATP (NU-1612-xxx)

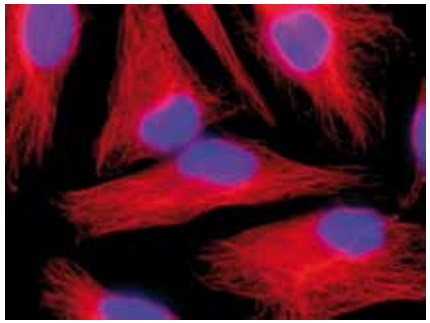
All above nucleotides are available with the ATTO-labels shown in the table below. They can be ordered directly from Jena Bioscience (www.jenabioscience.com) or via ATTO-TEC. Send an e-mail to sales@atto-tec.com and we will provide you with price information and lead time. To create the applicable order code, substitute for xxx the ATTO-dye number.

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 647**N**	ATTO 655
ATTO Oxa12	ATTO 665	ATTO 680	ATTO 700	ATTO 725
ATTO 740	ATTO MB2			

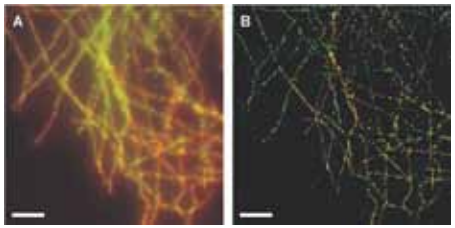


HeLa cells stained with LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Vendikar Yellow **ATTO 550** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.



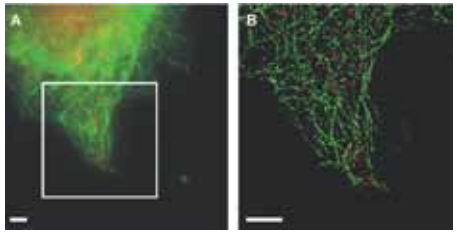
HeLa cells stained with LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Regulus Red **ATTO 594** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.

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.

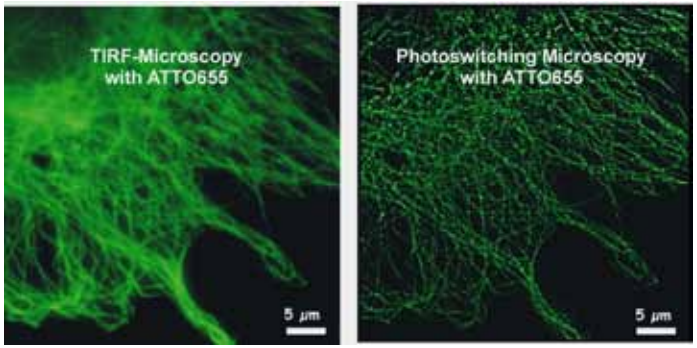


Dual-color photoswitching microscopy with **ATTO 520** and **ATTO 655** labeled microtubules in COS-7 cells. The reconstructed photoswitching image is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed sequentially in PBS, pH 7.4 in the presence 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).

M. Sauer et al., University of Würzburg.

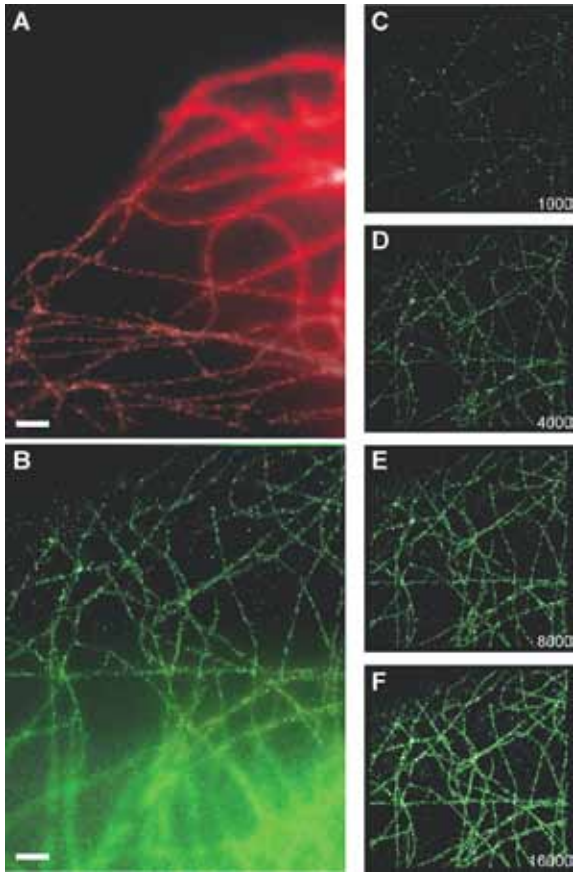


Dual-color photoswitching microscopy with **ATTO 520** labeled microtubules and **ATTO 655** labeled cytochrome c oxidase localized in the inner mitochondrial membrane of COS-7 cells. The reconstructed dual-color photoswitching image (expanded section) is shown in (B) and compared to the corresponding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



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.

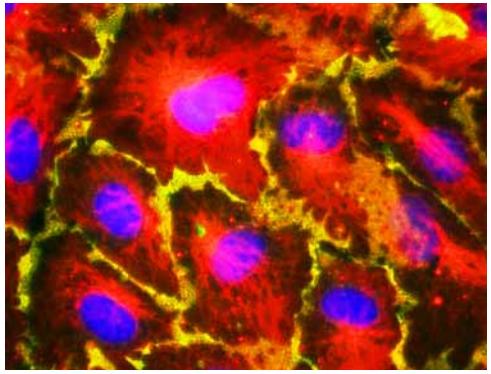
M. Sauer et al., University of Würzburg.



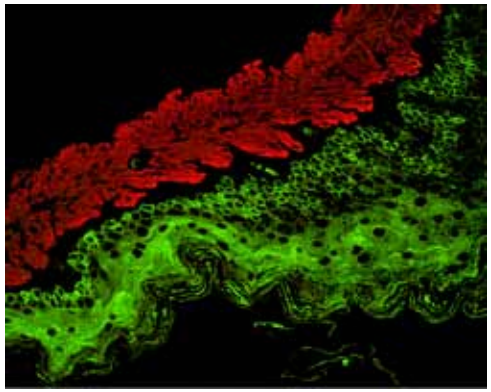
Photoswitching microscopy performed with the two standard fluorophores **ATTO 655** and **ATTO 520**. The upper right part of image A and lower part of image B are conventional immuno-fluorescence images of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** (A) and **ATTO 520** (B) labeled F(ab´)$_2$ fragments. Photoswitching microscopy images with subdiffraction resolution (scale bar 1 µm) are superimposed in the lower left (A) and upper (B) part of the images to visualize resolution improvement. Measurements were performed in PBS, pH 7.4 in the presence of 100 mM 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., University of Würzburg.

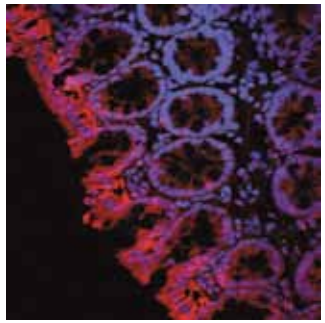
dSTORM super-resolution imaging of the cytoskeletal network of mammalian cells with different ATTO dyes spanning the visible wavelength range (from left to right: **ATTO 520, ATTO 565, ATTO 590, ATTO 655**, and **ATTO 700**). Immunofluorescence images (TIRF microscopy) of microtubules in COS-7 cells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



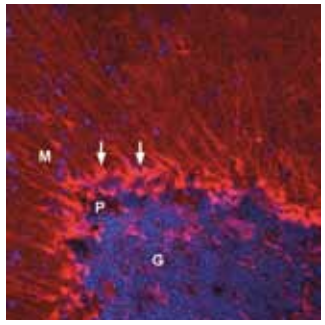
HUVEC: alpha-Tubulin/**ATTO 532**; E-Cadherin/**ATTO 655** and DAPI.



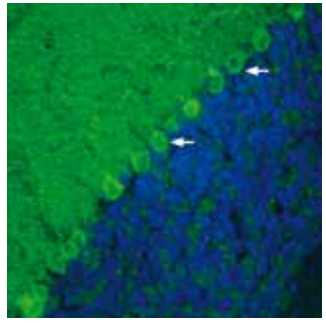
Rat stomach: Actin stained with mouse anti-smooth muscle α-actin antibody and **ATTO 488** anti-mouse IgG (green). Cytokeratin stained with polyclonal rabbit anti-cytokeratin and **ATTO 647N** anti-rabbit IgG (red).



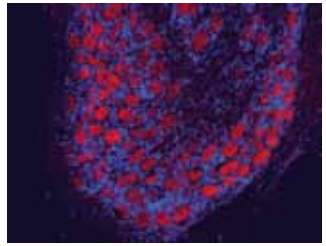
Expression of Aquaporin 3 in rat colon.
Rat colon sections (paraffin-embedded) were stained with Anti-Aquaporin 3-**ATTO-594** antibody (1:100). Staining (red color) is present in absorptive cells of the colonic epithelium. Hoechst 33342 (blue) is used as counterstain.



Expression of K_v1.5 in mouse cerebellum.
Immunohistochemical staining of perfusion fixed, free-floating frozen mouse brain sections using Anti-K_v1.5-**ATTO-550** (1:50). Staining (red) was detected in cerebellar Bergmann glial cells (white arrows). DAPI is used as the counterstain (blue). G = granule layer, P = Purkinje layer, M = molecular layer.



Expression of mGluR1 in rat cerebellum.
Immunohistochemical staining of rat cerebellum frozen sections using Anti-mGluR1 (extracellular)-**ATTO-488** antibody (1:20). Staining (green) appears in cerebellar Purkinje cells (arrows) and in the molecular layer.



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

List of Abbreviations

Abbreviation	
λ	wavelength
λ_{abs}	longest-wavelength absorption maximum
ε_{max}	molar decadic extinction coefficient at the longest-wavelength absorption maximum
ε_{260}	molar decadic extinction coefficient at λ = 260 nm
ε_{280}	molar decadic extinction coefficient at λ = 280 nm
CF_{260}	$CF_{260} = \varepsilon_{260}/\varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-DNA conjugates.
CF_{280}	$CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor used in the determination of degree of labeling (DOL) in case of dye-protein conjugates.
λ_{fl}	fluorescence maximum
η_{fl}	fluorescence quantum yield
τ_{fl}	real fluorescence decay time, $\tau_{fl} = \eta_{fl} \times \tau_0$
τ_0	natural (radiative) decay time
η_T	triplet quantum yield
MW	molecular weight
M^+	molecular weight of dye cation (HPLC-MS)
MH^+	molecular weight of protonated dye (HPLC-MS)
An^-	counterion(s)
Δm	increase of molecular mass on conjugation with ATTO-labels
Δq	change of electrical charge on conjugation with ATTO-labels
PBS	phosphate buffered saline
DOL	degree of labeling
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

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