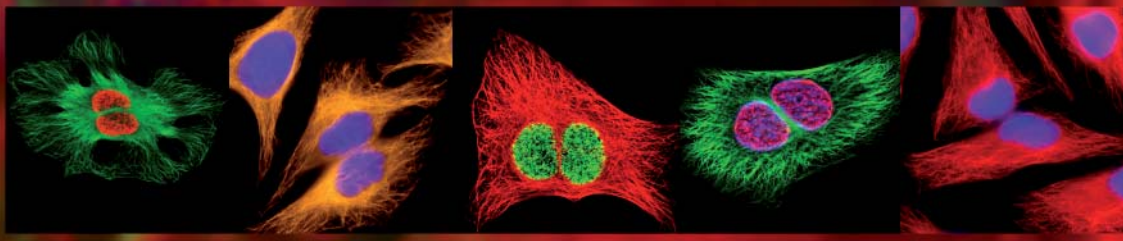
ATTO-TEC



# *Fluorescent Labels and Dyes*

***catalogue 2009/2010***

***Cover:***

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

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

# Contents

## ATTO-TEC GmbH
## Fluorescence - Our Passion

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

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

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

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

*The Team of* **ATTO-TEC**

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



ATTO-TEC

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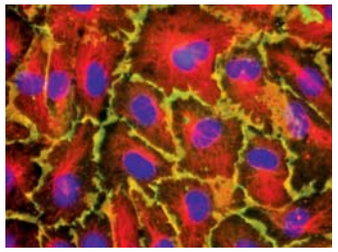

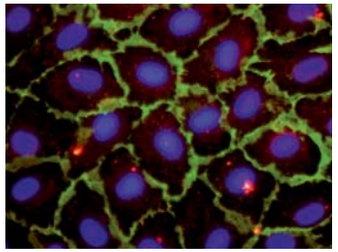

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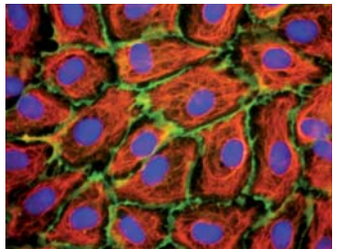


## Fluorescence

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. 50) has a high extinction coefficient at 635 nm (as follows from $\varepsilon_{max}$ and inspection of the absorption curve) as well as an excellent quantum yield of fluorescence ($\eta_{fl}$ = 0.65). It is to be noted, however, that besides optical considerations other factors may be important for the choice of label, e.g. pH-dependence, solubility, photostability, size of chromophore or linker and many others.

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

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

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

## Fluorescence Resonance Energy Transfer (FRET)

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

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

$N_A$ Avogadro constant
$n$ index of refraction
$\tau_0$ radiative decay time of donor
$r$ distance between donor and acceptor molecule
$F(\lambda)$ fluorescence spectrum of donor, normalized according $\int F(\lambda)\, d\lambda = 1$
$\varepsilon(\lambda)$ extinction coefficient of acceptor
$\kappa^2$ orientation factor: $\kappa^2 = (\cos\varphi_{DA} - 3\cos\varphi_D \cos\varphi_A)^2$
- $\varphi_{DA}$ angle between transition dipoles of donor and acceptor
- $\varphi_D$ angle between donor transition dipole and line connecting the dipoles
- $\varphi_A$ angle between acceptor transition dipole and line connecting the dipoles





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

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

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

A table of Förster-radii for ATTO-dyes is presented on p. 12-13. These values have been calculated with the assumption of statistical orientation of both donor and acceptor (orientation factor $\kappa^2 = 2/3$), a situation typically encountered in solutions of unbound dye molecules. However, in case of dye labeled biomolecules the chromophores of donor and acceptor may be held rigidly in a fixed position. As a consequence the orientation factor will assume a value different from 2/3. Since for $\kappa^2$ 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 | 520 | 532 | 540Q | 550 | 565 | 580Q | 590 | 594 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 390 | 14 | 41 | 50 | 58 | 59 | 60 | 56 | 54 | 53 | 52 | 47 | 48 | 45 |
| 425 | | 36 | 46 | 59 | 59 | 61 | 58 | 56 | 56 | 55 | 51 | 51 | 49 |
| 465 | | | 35 | 52 | 51 | 61 | 56 | 55 | 56 | 55 | 53 | 54 | 52 |
| 488 | | | | 50 | 46 | 61 | 64 | 63 | 63 | 63 | 60 | 60 | 57 |
| 495 | | | | | 41 | 56 | 56 | 56 | 57 | 58 | 55 | 56 | 54 |
| 520 | | | | | | 57 | 65 | 66 | 67 | 67 | 64 | 64 | 61 |
| 532 | | | | | | | 57 | 63 | 68 | 68 | 67 | 68 | 66 |
| 550 | | | | | | | | | 58 | 63 | 69 | 70 | 68 |
| 565 | | | | | | | | | | 61 | 69 | 72 | 71 |
| 590 | | | | | | | | | | | | 63 | 66 |
| 594 | | | | | | | | | | | | | 62 |
| 610 | | | | | | | | | | | | | |
| 611X | | | | | | | | | | | | | |
| 620 | | | | | | | | | | | | | |
| 633 | | | | | | | | | | | | | |
| 647 | | | | | | | | | | | | | |
| 647N | | | | | | | | | | | | | |
| 655 | | | | | | | | | | | | | |
| 680 | | | | | | | | | | | | | |
| 700 | | | | | | | | | | | | | |
| 725 | | | | | | | | | | | | | |
| 740 | | | | | | | | | | | | | |

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

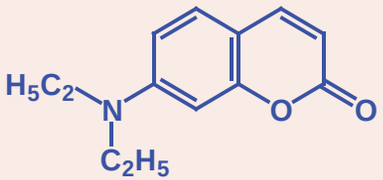
Introduction

## Molecular Structure of Fluorescent Labels

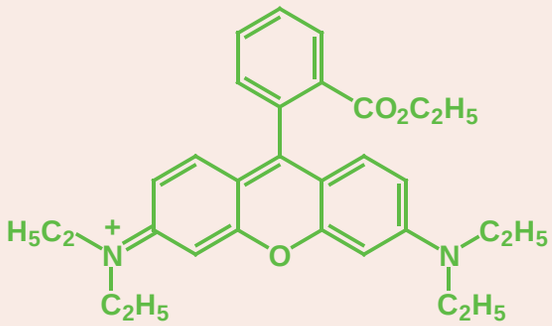
The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of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

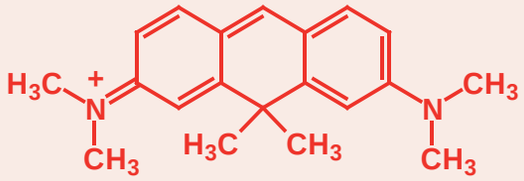
- **Coumarin**



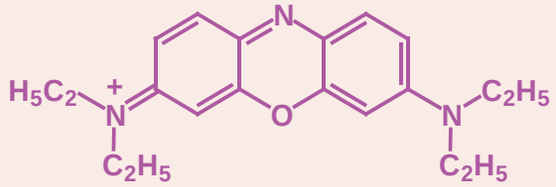

- **Rhodamine**




- **Carbopyronin**



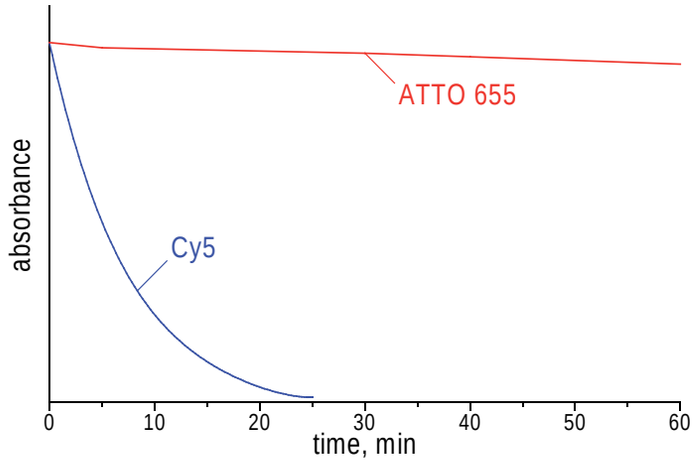

- **Oxazine**



Introduction

## Properties of Fluorescent Labels

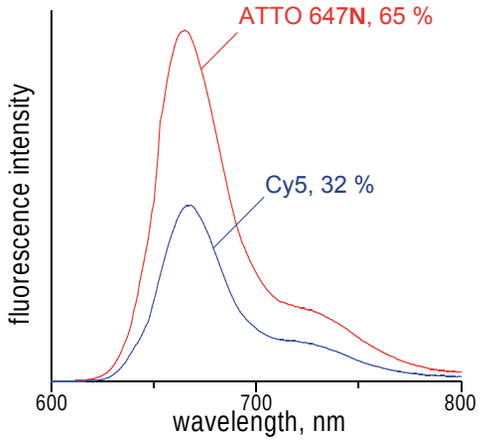
Apart from absorption and fluorescence there are many other properties that are highly relevant with respect to the suitability of dyes as labels. Most important, the dye must remain intact during irradiation. Many common labels, e.g. Fluorescein (FITC), show very low photostability. As a result sensitivity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

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

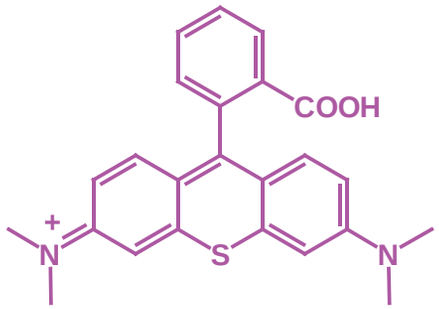
Besides reduced background, an advantage of excitation in the red spectral region is that rugged diode lasers are readily available. Diode lasers are generally less expensive and more energy-efficient than gas lasers. Furthermore a variety of sensitive detectors is available for the visible-near-IR region. 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. 28) and **ATTO 495** (p. 30), both absorbing below 500 nm, we supply **ATTO Thio12** (p. 39), a triplet label derived from *Thiorhodamine*.

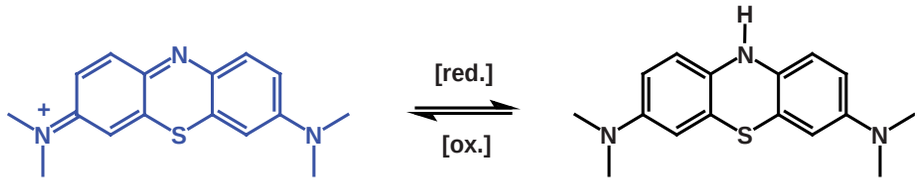



## Redox Labels

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








**Methylene Blue**
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. 59). The dye is further available as NHS-ester for direct coupling to amino groups or as maleimide derivative for coupling to thiol groups. ATTO-TEC also supplies a biotin conjugate for binding to avidin or streptavidin. Additional conjugates can be prepared on request.

## Reactive Labels and Conjugates

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

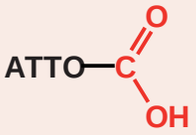
All ATTO-labels are available as NHS-esters for coupling to amino groups and as maleimides for coupling to thiol groups. In addition we offer most ATTO dyes functionalized with amine, azide (Click-Chemistry) and iodoacetamide. Dyes with other reactive substituents can be supplied on request.

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

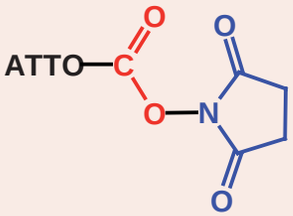
Introduction

## ATTO Derivatives and Conjugates

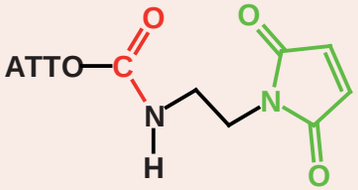
### ATTO-dye with free COOH:



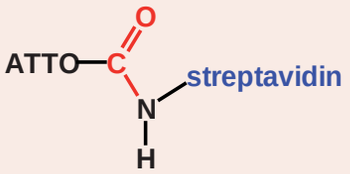

### NHS-ester:



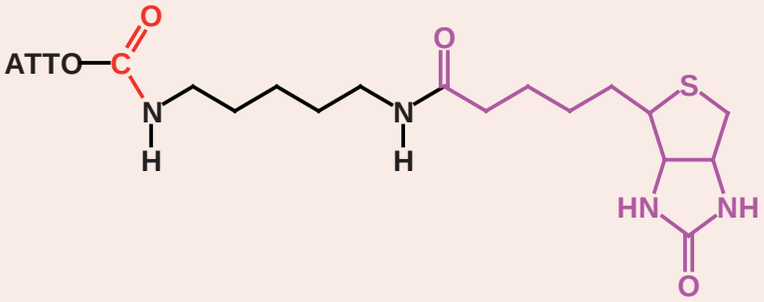

### Maleimide:



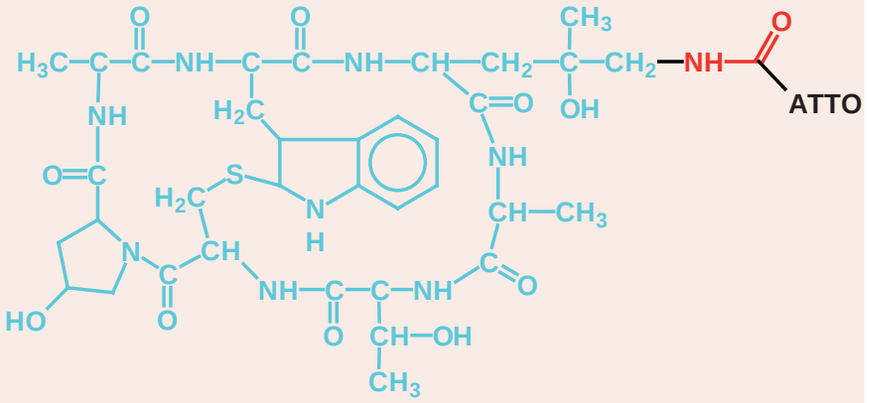

### Streptavidin conjugate:



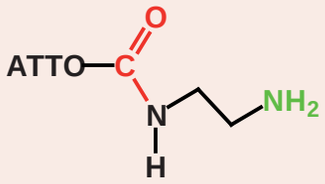

### Biotin conjugate:



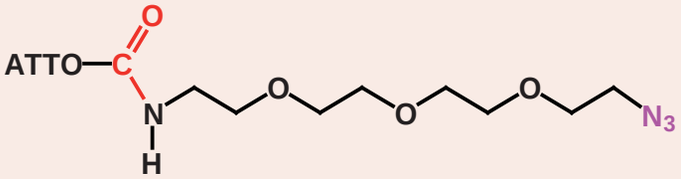

### Phalloidin conjugate:



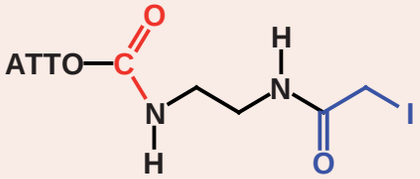
Amine:




Azide:




Iodoacetamide:






Introduction

## About this Catalogue

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

The spectra presented in this catalogue will help to select the dye best suited for a particular experiment. For accurate data in digitized form the reader is referred to www.atto-tec.com (*Products*, or *Support - Downloads - Datatable*). - The correction factors $CF_{260}$ and $CF_{280}$ aide with calculating the degree of labeling (DOL), see „Labeling Procedures“ (p. 68-73).

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

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

ATTO-Labels
Optical Properties

## ATTO Fluorescent Labels

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

## ATTO Fluorescence Quenchers

| Label | $\lambda_{abs}$, nm | $\varepsilon_{max}$, $M^{-1}$ $cm^{-1}$ | 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 | $\lambda_{abs}$, nm | $\varepsilon_{max}$, $M^{-1}$ $cm^{-1}$ | $\lambda_{fl}$, nm | $\eta_{fl}$, % |
|---|---|---|---|---|
| ATTO 390 | 390 | 24000 | 479 | 90 |
| ATTO 425 | 436 | 45000 | 484 | 90 |
| ATTO 465 | 453 | 75000 | 508 | 55 |
| ATTO 611X | 611 | 100000 | 681 | 35 |

## ATTO Triplet Label

| Label | $\lambda_{abs}$, nm | $\varepsilon_{max}$, $M^{-1}$ $cm^{-1}$ | $\lambda_{fl}$, nm | $\eta_T$, % |
|---|---|---|---|---|
| ATTO 465 | 453 | 75000 | 508 | 10 |
| ATTO 495 | 495 | 80000 | 527 | 10 |
| ATTO Thio12 | 579 | 110000 | 609 | 20 |

## ATTO Redox Label

| Label | $\lambda_{abs}$, nm | $\varepsilon_{max}$, $M^{-1}$ $cm^{-1}$ |
|---|---|---|
| ATTO MB2 | 658 | 110000 |

| | |
|---|---|
| $\lambda_{abs}$ | longest-wavelength absorption maximum |
| $\varepsilon_{max}$ | molar extinction coefficient at the longest-wavelength absorption maximum |
| $\lambda_{fl}$ | fluorescence maximum |
| $\eta_{fl}$ | fluorescence quantum yield |
| $\tau_{fl}$ | fluorescence decay time |
| $\tau_0$ | natural (radiative) fluorescence decay time |
| MW | molecular weight |
| $M^+$ | molecular weight of dye cation (HPLC-MS) |
| $MH^+$ | molecular weight of protonated dye (HPLC-MS) |
| $CF_{260}$ | $CF_{260} = \varepsilon_{260}/\varepsilon_{max}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-DNA conjugates. |
| $CF_{280}$ | $CF_{280} = \varepsilon_{280}/\varepsilon_{max}$. Correction factor used in calculation of degree of labeling (DOL) in case of dye-protein conjugates. |

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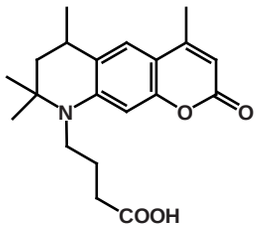
## ATTO 390

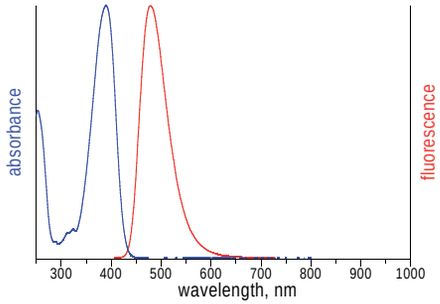
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 390 nm | | |
| $\varepsilon_{max}$ | = | 2.4 x $10^4$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 479 nm | | |
| $\eta_{fl}$ | = | 90 % | $CF_{260}$ = | 0.52 |
| $\tau_{fl}$ | = | 3.8 ns | $CF_{280}$ = | 0.08 |

*Features:*

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




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

* 10 nmol **20 nmol

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



Fluorescent Labels
350 nm - 500 nm

ATTO-TEC

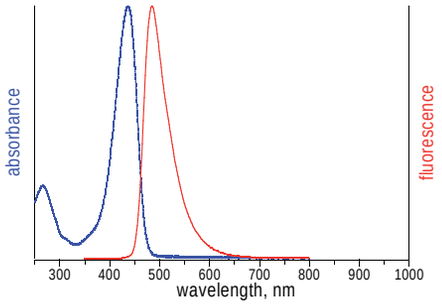
## ATTO 425

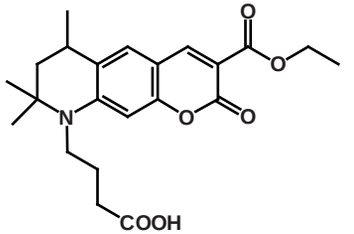
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 436 nm | | |
| $\varepsilon_{max}$ | = | 4.5 x $10^4$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 484 nm | | |
| $\eta_{fl}$ | = | 90 % | $CF_{260}$ = | 0.27 |
| $\tau_{fl}$ | = | 3.5 ns | $CF_{280}$ = | 0.23 |

*Features:*

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




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

* 10 nmol **20 nmol

Other conjugates with sec. antibodies etc. on request.

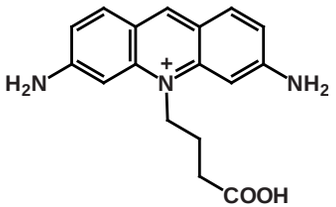
## ATTO 465

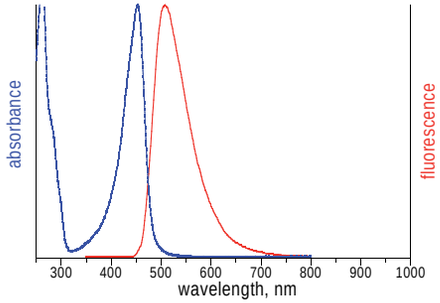
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 453 nm | | |
| $\varepsilon_{max}$ | = | 7.5 x $10^4$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 508 nm | | |
| $\eta_{fl}$ | = | 55 % | $CF_{260}$ = | 1.12 |
| $\tau_{fl}$ | = | 2.2 ns | $CF_{280}$ = | 0.54 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 396 | 296 | AD 465-21 | AD 465-25 |
| NHS-ester | 493 | 393 | AD 465-31 | AD 465-35 |
| maleimide | 518 | 418 | AD 465-41 | AD 465-45 |
| streptavidin | | | AD 465-61 | AD 465-65 |
| biotin | 706 | 606 | AD 465-71 | AD 465-75 |

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





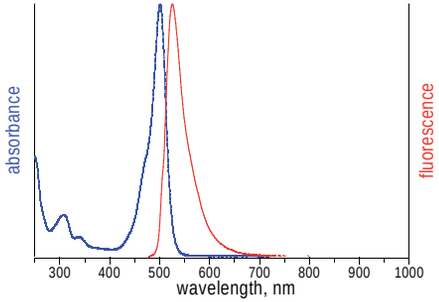
## ATTO 488

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 501 nm | | |
| $\varepsilon_{max}$ | = | 9.0 x $10^4$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 523 nm | | |
| $\eta_{fl}$ | = | 80 % | $CF_{260}$ = | 0.25 |
| $\tau_{fl}$ | = | 3.2 ns | $CF_{280}$ = | 0.10 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 804 | 590 | AD 488-21 | AD 488-25 |
| NHS-ester | 981 | 687 | AD 488-31 | AD 488-35 |
| maleimide | 1067 | 712 | AD 488-41 | AD 488-45 |
| streptavidin | | | AD 488-61 | AD 488-65 |
| biotin | 1191 | 900 | AD 488-71 | AD 488-75 |
| phalloidin | 1472 | 1359 | AD 488-81* | AD 488-82** |
| amine | 858 | 632 | AD 488-91 | AD 488-95 |
| azide | 903 | 790 | AD 488-101 | AD 488-105 |
| iodoacetamide | 913 | 800 | AD 488-111 | AD 488-115 |

* 10 nmol **20 nmol

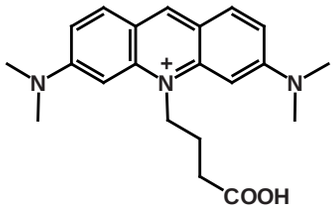
# ATTO 495

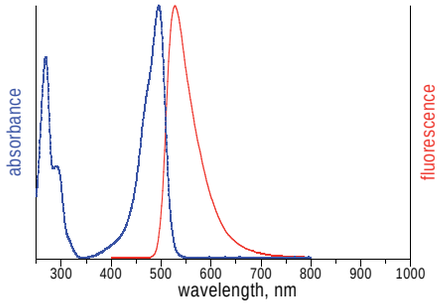
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 495 nm | | |
| $\varepsilon_{max}$ | = | 8.0 x $10^4$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 527 nm | | |
| $\eta_{fl}$ | = | 45 % | $CF_{260}$ = | 0.57 |
| $\tau_{fl}$ | = | 2.4 ns | $CF_{280}$ = | 0.39 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 452 | 352 | AD 495-21 | AD 495-25 |
| NHS-ester | 549 | 449 | AD 495-31 | AD 495-35 |
| maleimide | 574 | 474 | AD 495-41 | AD 495-45 |
| streptavidin | | | AD 495-61 | AD 495-65 |
| biotin | 762 | 662 | AD 495-71 | AD 495-75 |

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





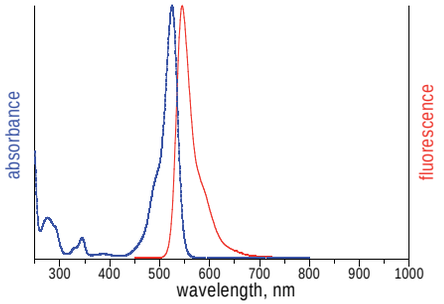
# ATTO 520

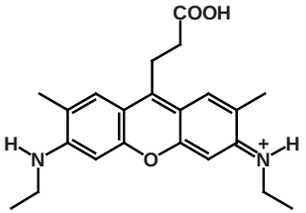
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 516 nm | | |
| $\varepsilon_{max}$ | = | 1.1 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 538 nm | | |
| $\eta_{fl}$ | = | 90 % | $CF_{260}$ = | 0.13 |
| $\tau_{fl}$ | = | 3.8 ns | $CF_{280}$ = | 0.18 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 467 | 367 | AD 520-21 | AD 520-25 |
| NHS-ester | 564 | 464 | AD 520-31 | AD 520-35 |
| maleimide | 589 | 489 | AD 520-41 | AD 520-45 |
| biotin | 777 | 677 | AD 520-71 | AD 520-75 |

Other conjugates with phalloidin etc. on request.

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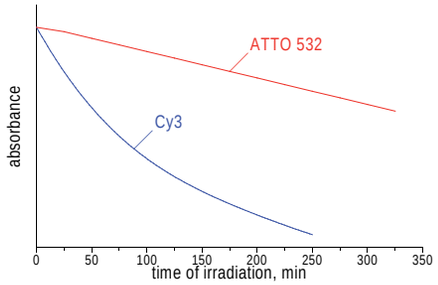
## ATTO 532

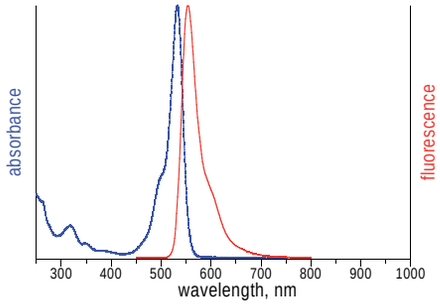
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 532 nm | | |
| $\varepsilon_{max}$ | = | 1.15 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 553 nm | | |
| $\eta_{fl}$ | = | 90 % | $CF_{260}$ = | 0.22 |
| $\tau_{fl}$ | = | 3.8 ns | $CF_{280}$ = | 0.11 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 765 | 646 | AD 532-21 | AD 532-25 |
| NHS-ester | 1081 | 743 | AD 532-31 | AD 532-35 |
| maleimide | 1063 | 768 | AD 532-41 | AD 532-45 |
| streptavidin | | | AD 532-61 | AD 532-65 |
| biotin | 1357 | 956 | AD 532-71 | AD 532-75 |
| phalloidin | 1530 | 1417 | AD 532-81* | AD 532-82** |
| amine | 914 | 688 | AD 532-91 | AD 532-95 |
| azide | 959 | 846 | AD 532-101 | AD 532-105 |
| iodoacetamide | 969 | 856 | AD 532-111 | AD 532-115 |

* 10 nmol **20 nmol



Fluorescent Labels
500 nm - 600 nm

ATTO-TEC

## ATTO Rho6G

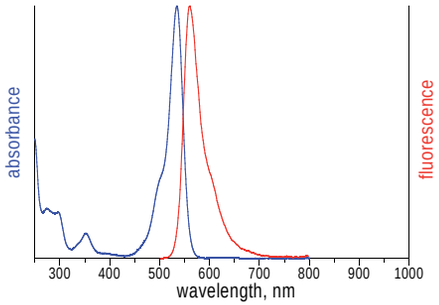
New

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 535 nm | | |
| $\varepsilon_{max}$ | = | 1.15 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 560 nm | $CF_{260}$ = | 0.22 |
| $\eta_{fl}$ | = | 90 % | $CF_{280}$ = | 0.19 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 614 | 514 | AD Rho6G-21 | AD Rho6G-25 |
| NHS-ester | 711 | 611 | AD Rho6G-31 | AD Rho6G-35 |
| maleimide | 749 | 636 | AD Rho6G-41 | AD Rho6G-45 |
| biotin | 937 | 824 | AD Rho6G-71 | AD Rho6G-75 |
| phalloidin | 1396 | 1283 | AD Rho6G-81* | AD Rho6G-82** |

* 10 nmol **20 nmol

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

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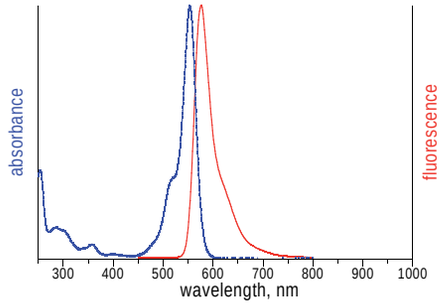
## ATTO 550

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 554 nm
$\varepsilon_{max}$ = 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$
$\lambda_{fl}$ = 576 nm
$\eta_{fl}$ = 80 %
$\tau_{fl}$ = 3.2 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 Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 694 | 594 | AD 550-21 | AD 550-25 |
| NHS-ester | 791 | 691 | AD 550-31 | AD 550-35 |
| maleimide | 816 | 716 | AD 550-41 | AD 550-45 |
| streptavidin | | | AD 550-61 | AD 550-65 |
| biotin | 1004 | 904 | AD 550-71 | AD 550-75 |
| phalloidin | 1476 | 1363 | AD 550-81* | AD 550-82** |
| amine | 862 | 636 | AD 550-91 | AD 550-95 |
| azide | 907 | 794 | AD 550-101 | AD 550-105 |
| iodoacetamide | 917 | 804 | AD 550-111 | AD 550-115 |

* 10 nmol **20 nmol



Fluorescent Labels
500 nm - 600 nm

ATTO-TEC

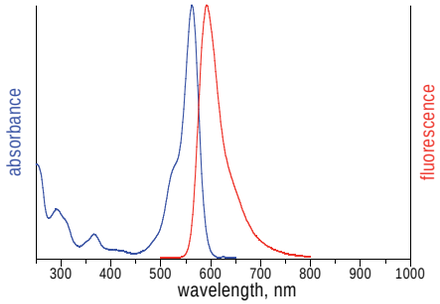
## ATTO 565

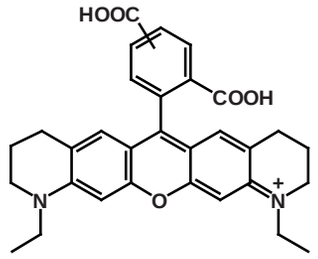
**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 563 nm
$\varepsilon_{max}$ = 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$
$\lambda_{fl}$ = 592 nm
$\eta_{fl}$ = 90 %
$\tau_{fl}$ = 3.4 ns
$CF_{260}$ = 0.34
$CF_{280}$ = 0.16

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 611 | 511 | AD 565-21 | AD 565-25 |
| NHS-ester | 708 | 608 | AD 565-31 | AD 565-35 |
| maleimide | 733 | 633 | AD 565-41 | AD 565-45 |
| streptavidin | | | AD 565-61 | AD 565-65 |
| biotin | 921 | 821 | AD 565-71 | AD 565-75 |
| phalloidin | 1393 | 1280 | AD 565-81* | AD 565-82** |
| amine | 666 | 553 | AD 565-91 | AD 565-95 |
| azide | 824 | 711 | AD 565-101 | AD 565-105 |
| iodoacetamide | 834 | 721 | AD 565-111 | AD 565-115 |

* 10 nmol **20 nmol

## ATTO Rho3B

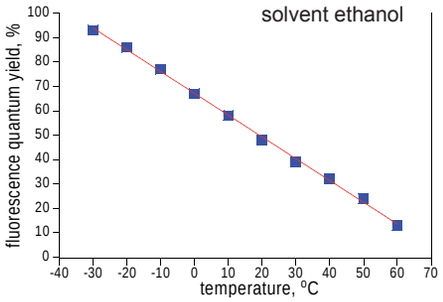
New

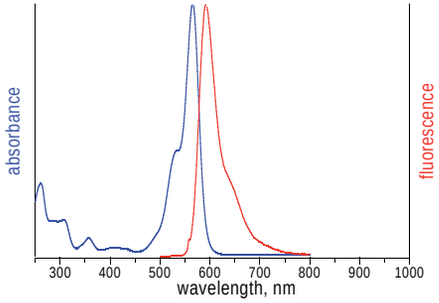
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 565 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 592 nm | $CF_{260}$ = | 0.28 |
| $\eta_{fl}$ | = | 50 % (in ethanol, 20°C) | $CF_{280}$ = | 0.14 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 642 | 542 | AD Rho3B-21 | AD Rho3B-25 |
| NHS-ester | 739 | 639 | AD Rho3B-31 | AD Rho3B-35 |
| maleimide | 764 | 664 | AD Rho3B-41 | AD Rho3B-45 |
| biotin | 965 | 852 | AD Rho3B-71 | AD Rho3B-75 |

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



Fluorescent Labels
500 nm - 600 nm

## ATTO Rho11

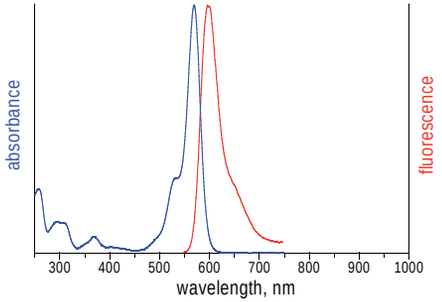
New

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 571 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 595 nm | $CF_{260}$ = | 0.25 |
| $\eta_{fl}$ | = | 80 % | $CF_{280}$ = | 0.09 |

*Features:*

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




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

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

## ATTO Rho12

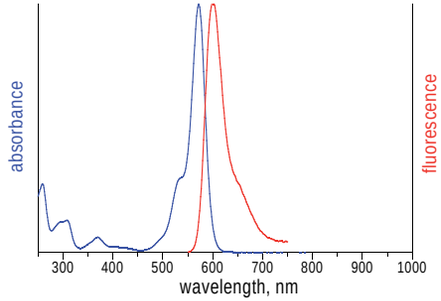
New

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 576 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 601 nm | $CF_{260}$ = | 0.27 |
| $\eta_{fl}$ | = | 80 % | $CF_{280}$ = | 0.09 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 750 | 650 | AD Rho12-21 | AD Rho12-25 |
| NHS-ester | 847 | 747 | AD Rho12-31 | AD Rho12-35 |
| maleimide | 872 | 772 | AD Rho12-41 | AD Rho12-45 |
| biotin | 1073 | 960 | AD Rho12-71 | AD Rho12-75 |

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





## ATTO Thio12

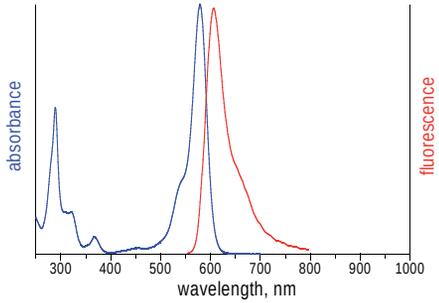
New

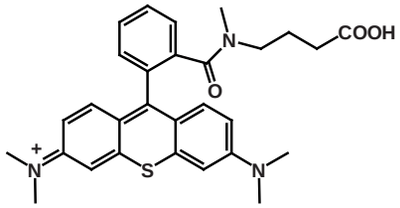
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 579 nm | | |
| $\varepsilon_{max}$ | = | 1.1 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 609 nm | | |
| $\eta_{fl}$ | = | 15 % | $CF_{260}$ = | 0.10 |
| $\eta_T$ | = | 20 % | $CF_{280}$ = | 0.37 |

*Features:*

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




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

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

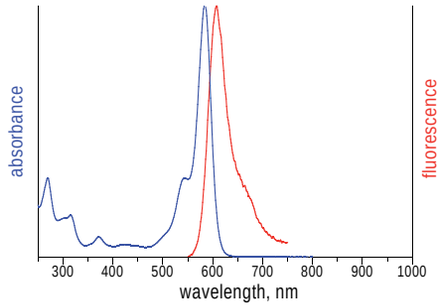
## ATTO Rho101 New

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 586 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 610 nm | $CF_{260}$ = | 0.24 |
| $\eta_{fl}$ | = | 80 % | $CF_{280}$ = | 0.19 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 703 | 590 | AD Rho101-21 | AD Rho101-25 |
| NHS-ester | 787 | 687 | AD Rho101-31 | AD Rho101-35 |
| maleimide | 812 | 712 | AD Rho101-41 | AD Rho101-45 |
| biotin | 1013 | 900 | AD Rho101-71 | AD Rho101-75 |

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



Fluorescent Labels
500 nm - 600 nm

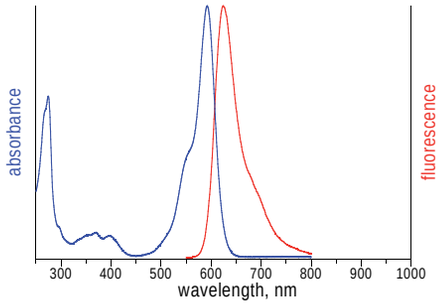
## ATTO 590

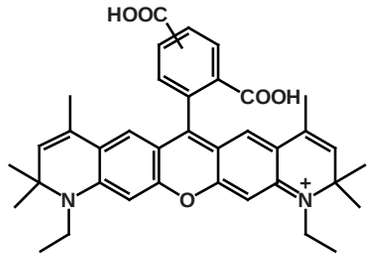
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 594 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 624 nm | | |
| $\eta_{fl}$ | = | 80 % | $CF_{260}$ = | 0.42 |
| $\tau_{fl}$ | = | 3.7 ns | $CF_{280}$ = | 0.44 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 691 | 591 | AD 590-21 | AD 590-25 |
| NHS-ester | 788 | 688 | AD 590-31 | AD 590-35 |
| maleimide | 813 | 713 | AD 590-41 | AD 590-45 |
| streptavidin | | | AD 590-61 | AD 590-65 |
| biotin | 1001 | 901 | AD 590-71 | AD 590-75 |
| phalloidin | 1473 | 1360 | AD 590-81* | AD 590-82** |
| amine | 916 | 689 | AD 590-91 | AD 590-95 |
| azide | 904 | 791 | AD 590-101 | AD 590-105 |
| iodoacetamide | 970 | 857 | AD 590-111 | AD 590-115 |

* 10 nmol **20 nmol

ATTO-TEC

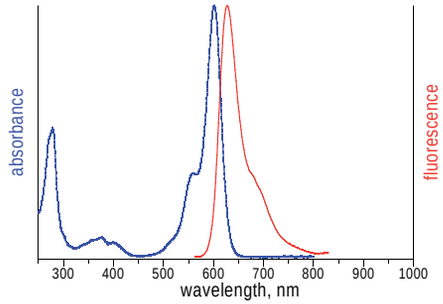
## ATTO 594

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 601 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 627 nm | | |
| $\eta_{fl}$ | = | 85 % | $CF_{260}$ = | 0.26 |
| $\tau_{fl}$ | = | 3.5 ns | $CF_{280}$ = | 0.51 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 1137 | 806 | AD 594-21 | AD 594-25 |
| NHS-ester | 1389 | 903 | AD 594-31 | AD 594-35 |
| maleimide | 1358 | 928 | AD 594-41 | AD 594-45 |
| streptavidin | | | AD 594-61 | AD 594-65 |
| biotin | 1456 | 1116 | AD 594-71 | AD 594-75 |
| amine | 1174 | 948 | AD 594-91 | AD 594-95 |

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



Fluorescent Labels
500 nm - 600 nm

ATTO-TEC

New

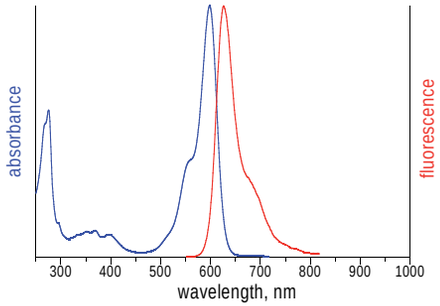
## ATTO Rho13

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 600 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 625 nm | $CF_{260}$ = | 0.10 |
| $\eta_{fl}$ | = | 80 % | $CF_{280}$ = | 0.37 |

*Features:*

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




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

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

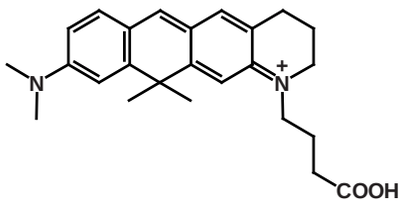
## ATTO 610

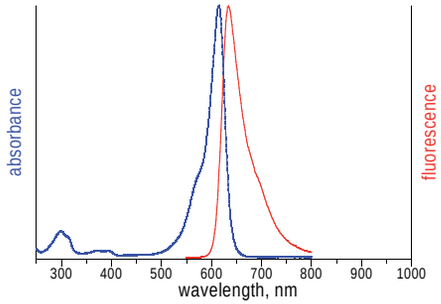
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 615 nm | | |
| $\varepsilon_{max}$ | = | 1.5 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 634 nm | | |
| $\eta_{fl}$ | = | 70 % | $CF_{260}$ = | 0.02 |
| $\tau_{fl}$ | = | 3.3 ns | $CF_{280}$ = | 0.05 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 491 | 391 | AD 610-21 | AD 610-25 |
| NHS-ester | 588 | 488 | AD 610-31 | AD 610-35 |
| maleimide | 613 | 513 | AD 610-41 | AD 610-45 |
| biotin | 801 | 701 | AD 610-71 | AD 610-75 |

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





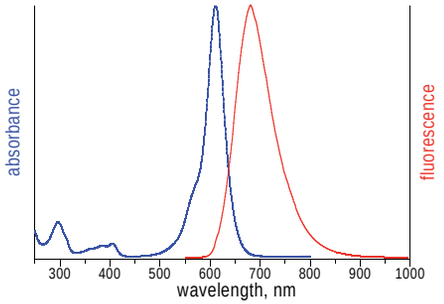
## ATTO 611X

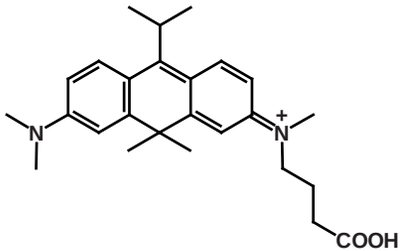
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 611 nm | | |
| $\varepsilon_{max}$ | = | 1.0 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 681 nm | | |
| $\eta_{fl}$ | = | 35 % | $CF_{260}$ = | 0.05 |
| $\tau_{fl}$ | = | 2.5 ns | $CF_{280}$ = | 0.07 |

*Features:*

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




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

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

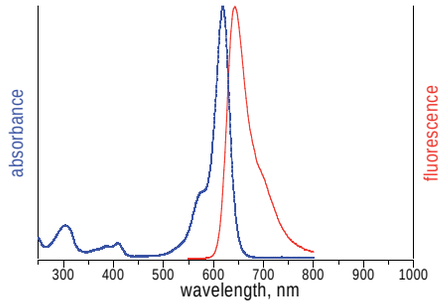
## ATTO 620

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 619 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 643 nm | | |
| $\eta_{fl}$ | = | 50 % | $CF_{260}$ = | 0.05 |
| $\tau_{fl}$ | = | 2.9 ns | $CF_{280}$ = | 0.07 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 612 | 512 | AD 620-21 | AD 620-25 |
| NHS-ester | 709 | 609 | AD 620-31 | AD 620-35 |
| maleimide | 734 | 634 | AD 620-41 | AD 620-45 |
| biotin | 922 | 822 | AD 620-71 | AD 620-75 |

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



Fluorescent Labels
600 nm - 700 nm

## ATTO Rho14

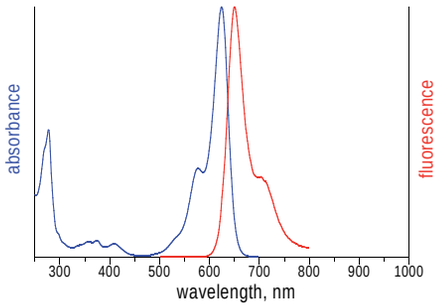
New

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 625 nm | | |
| $\varepsilon_{max}$ | = | 1.4 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 646 nm | $CF_{260}$ = | 0.29 |
| $\eta_{fl}$ | = | 80 % | $CF_{280}$ = | 0.46 |

*Features:*

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




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

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

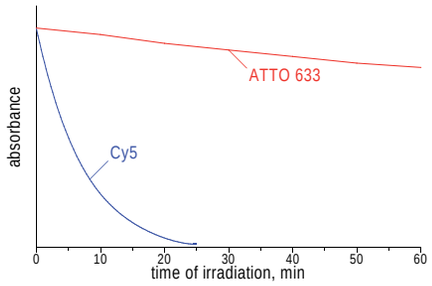
## ATTO 633

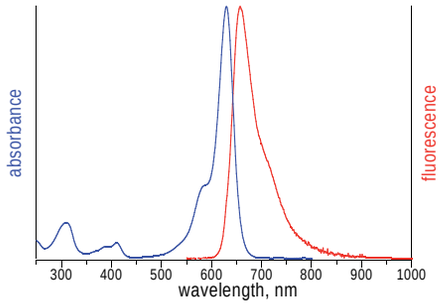
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 629 nm | | |
| $\varepsilon_{max}$ | = | 1.3 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 657 nm | | |
| $\eta_{fl}$ | = | 64 % | $CF_{260}$ = | 0.05 |
| $\tau_{fl}$ | = | 3.2 ns | $CF_{280}$ = | 0.06 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 652 | 552 | AD 633-21 | AD 633-25 |
| NHS-ester | 749 | 649 | AD 633-31 | AD 633-35 |
| maleimide | 774 | 674 | AD 633-41 | AD 633-45 |
| streptavidin | | | AD 633-61 | AD 633-65 |
| biotin | 962 | 862 | AD 633-71 | AD 633-75 |
| phalloidin | 1434 | 1321 | AD 633-81* | AD 633-82** |
| amine | 707 | 594 | AD 633-91 | AD 633-95 |
| azide | 865 | 752 | AD 633-101 | AD 633-105 |
| iodoacetamide | 875 | 762 | AD 633-111 | AD 633-115 |

* 10 nmol **20 nmol





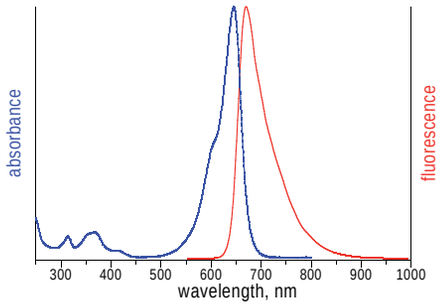
## ATTO 647

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 645 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 669 nm | | |
| $\eta_{fl}$ | = | 20 % | $CF_{260}$ = | 0.08 |
| $\tau_{fl}$ | = | 2.3 ns | $CF_{280}$ = | 0.04 |

*Features:*

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




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

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

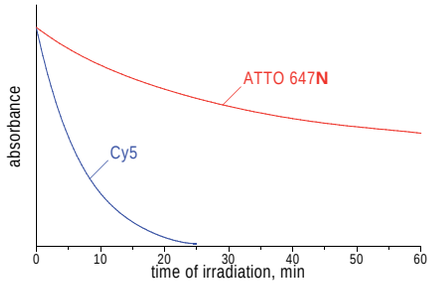
## ATTO 647N

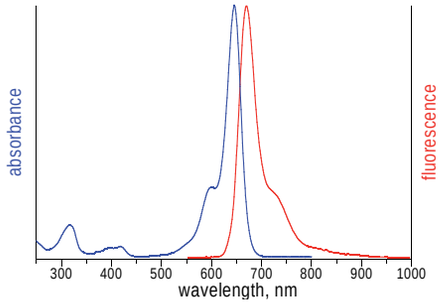
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 644 nm | | |
| $\varepsilon_{max}$ | = | 1.5 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 669 nm | | |
| $\eta_{fl}$ | = | 65 % | $CF_{260}$ = | 0.06 |
| $\tau_{fl}$ | = | 3.4 ns | $CF_{280}$ = | 0.05 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (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 |

* 10 nmol **20 nmol





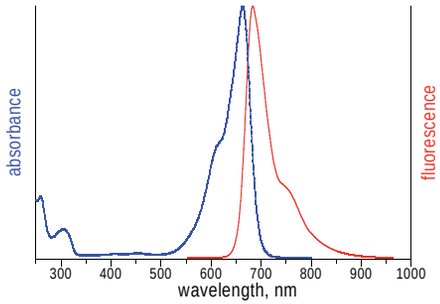
## ATTO 655

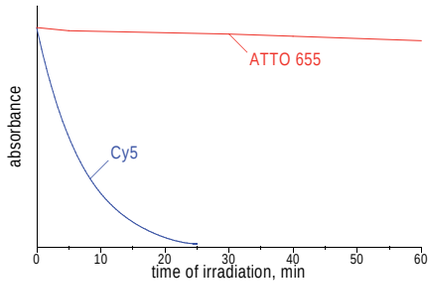
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 663 nm | | |
| $\varepsilon_{max}$ | = | 1.25 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 684 nm | | |
| $\eta_{fl}$ | = | 30 % | $CF_{260}$ = | 0.24 |
| $\tau_{fl}$ | = | 1.9 ns | $CF_{280}$ = | 0.08 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 634 | 528 | AD 655-21 | AD 655-25 |
| NHS-ester | 887 | 625 | AD 655-31 | AD 655-35 |
| maleimide | 812 | 650 | AD 655-41 | AD 655-45 |
| streptavidin | | | AD 655-61 | AD 655-65 |
| biotin | 1204 | 838 | AD 655-71 | AD 655-75 |
| phalloidin | 1410 | 1297 | AD 655-81* | AD 655-82** |
| amine | 796 | 570 | AD 655-91 | AD 655-95 |
| azide | 841 | 728 | AD 655-101 | AD 655-105 |
| iodoacetamide | 851 | 738 | AD 655-111 | AD 655-115 |

* 10 nmol **20 nmol

## ATTO Oxa12

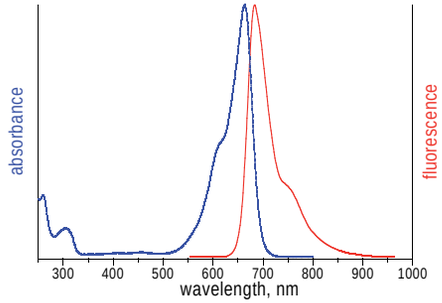
New

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 663 nm
$\varepsilon_{max}$ = 1.25 x $10^5$ $M^{-1}$ $cm^{-1}$
$\lambda_{fl}$ = 684 nm
$\eta_{fl}$ = 30 %
$CF_{260}$ = 0.24
$CF_{280}$ = 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 Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 738 | 639 | AD Oxa12-21 | AD Oxa12-25 |
| NHS-ester | 835 | 736 | AD Oxa12-31 | AD Oxa12-35 |
| maleimide | 874 | 761 | AD Oxa12-41 | AD Oxa12-45 |
| biotin | 1062 | 949 | AD Oxa12-71 | AD Oxa12-75 |

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



Fluorescent Labels
600 nm - 700 nm

## ATTO 665

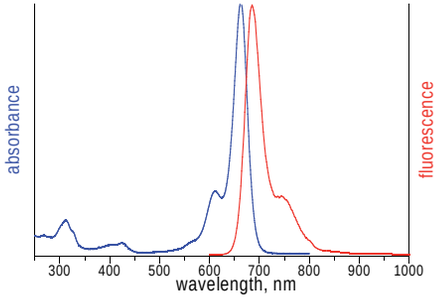
New

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 663 nm
$\varepsilon_{max}$ = 1.60 x $10^5$ $M^{-1}$ $cm^{-1}$
$\lambda_{fl}$ = 684 nm
$\eta_{fl}$ = 60 %
$CF_{260}$ = 0.07
$CF_{280}$ = 0.06

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 723 | 623 | AD 665-21 | AD 665-25 |
| NHS-ester | 820 | 720 | AD 665-31 | AD 665-35 |
| maleimide | 845 | 745 | AD 665-41 | AD 665-45 |
| biotin | 1046 | 933 | AD 665-71 | AD 665-75 |

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

ATTO-TEC

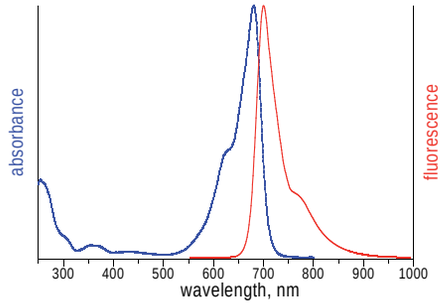
## ATTO 680

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 680 nm | | |
| $\varepsilon_{max}$ | = | 1.25 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 700 nm | | |
| $\eta_{fl}$ | = | 30 % | $CF_{260}$ = | 0.30 |
| $\tau_{fl}$ | = | 1.8 ns | $CF_{280}$ = | 0.17 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 631 | 526 | AD 680-21 | AD 680-25 |
| NHS-ester | 828 | 623 | AD 680-31 | AD 680-35 |
| maleimide | 1024 | 648 | AD 680-41 | AD 680-45 |
| streptavidin | | | AD 680-61 | AD 680-65 |
| biotin | 1123 | 836 | AD 680-71 | AD 680-75 |

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





ATTO-TEC

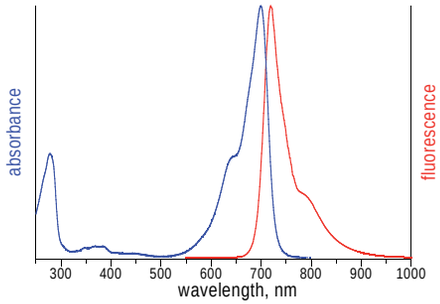
## ATTO 700

**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 700 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 719 nm | | |
| $\eta_{fl}$ | = | 25 % | $CF_{260}$ = | 0.26 |
| $\tau_{fl}$ | = | 1.5 ns | $CF_{280}$ = | 0.41 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 575 | 566 | AD 700-21 | AD 700-25 |
| NHS-ester | 837 | 663 | AD 700-31 | AD 700-35 |
| maleimide | 971 | 688 | AD 700-41 | AD 700-45 |
| streptavidin | | | AD 700-61 | AD 700-65 |
| biotin | 973 | 876 | AD 700-71 | AD 700-75 |
| amine | 722 | 608 | AD 700-91 | AD 700-95 |

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

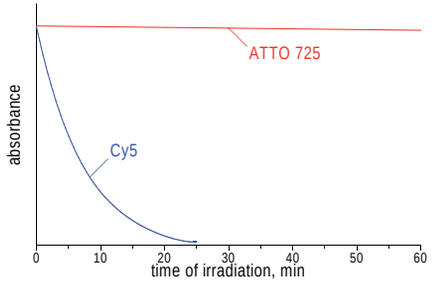
## ATTO 725

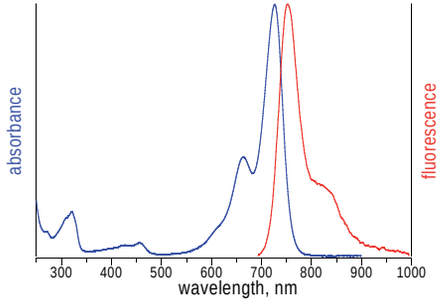
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 729 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 752 nm | | |
| $\eta_{fl}$ | = | 10 % | $CF_{260}$ = | 0.10 |
| $\tau_{fl}$ | = | 0.5 ns | $CF_{280}$ = | 0.08 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 516 | 416 | AD 725-21 | AD 725-25 |
| NHS-ester | 613 | 513 | AD 725-31 | AD 725-35 |
| maleimide | 638 | 538 | AD 725-41 | AD 725-45 |
| biotin | 826 | 726 | AD 725-71 | AD 725-75 |

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





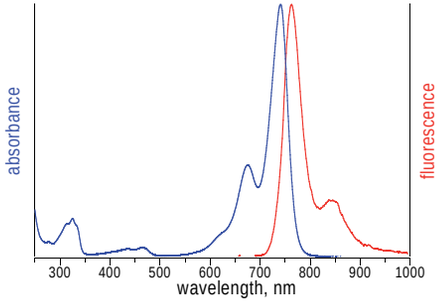
## ATTO 740

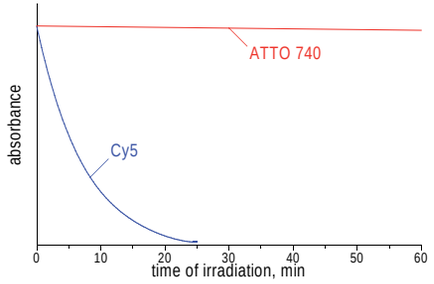
**Optical properties of carboxy derivative**

| | | | | |
|---|---|---|---|---|
| $\lambda_{abs}$ | = | 740 nm | | |
| $\varepsilon_{max}$ | = | 1.2 x $10^5$ $M^{-1}$ $cm^{-1}$ | | |
| $\lambda_{fl}$ | = | 764 nm | | |
| $\eta_{fl}$ | = | 10 % | $CF_{260}$ = | 0.11 |
| $\tau_{fl}$ | = | 0.6 ns | $CF_{280}$ = | 0.10 |

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 568 | 468 | AD 740-21 | AD 740-25 |
| NHS-ester | 665 | 565 | AD 740-31 | AD 740-35 |
| maleimide | 690 | 590 | AD 740-41 | AD 740-45 |
| biotin | 878 | 778 | AD 740-71 | AD 740-75 |

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

## Redox Label

A dye, well-known in biochemical and medical research, is *Methylene Blue*. It has very interesting redox properties: The dye, normally deep blue in color, is converted by mild reducing agents to its so-called *leuko*-form, which is colorless. Since this reaction is reversible, the blue color reappears on oxidation, e.g. by oxygen (air). These interconversions can be catalyzed enzymatically.
Methylene Blue as such cannot be coupled to biomolecules, because it lacks the necessary reactive groups. However, **ATTO-TEC** now offers **ATTO MB2**, a derivative of Methylene Blue. The dye is available as NHS-ester or maleimide for coupling to amino- or thiol groups, respectively. **ATTO MB2** is also supplied as biotin conjugate for direct coupling to avidin or streptavidin.





New

## ATTO MB2

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 658 nm

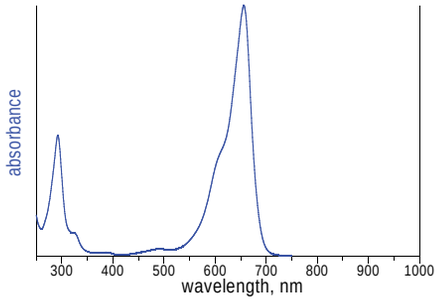
$\varepsilon_{max}$ = 1.00 x $10^5$ $M^{-1}$ $cm^{-1}$

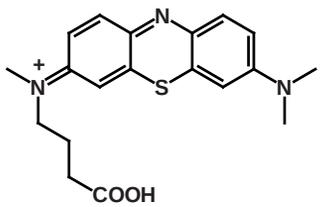
$CF_{260}$ = 0.11

$CF_{280}$ = 0.28

*Features:*

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




| Modification | MW, g/mol | $M^+$, g/mol | Order Code Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 392 | 356 | AD MB2-21 | AD MB2-25 |
| NHS-ester | 553 | 453 | AD MB2-31 | AD MB2-35 |
| maleimide | 591 | 478 | AD MB2-41 | AD MB2-45 |
| biotin | 779 | 666 | AD MB2-71 | AD MB2-75 |

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

Fluorescence resonance energy transfer (FRET) from an excited dye molecule (donor) to another nearby dye molecule (acceptor) leads to deactivation of the donor, i.e. it no longer fluoresces: Its fluorescence is *quenched*. The process of FRET depends, among other factors, on the absorption spectrum of the acceptor, as was discussed in some detail on p. 10-11. If the acceptor is *fluorescent* itself, it will emit light just the same, as if it had been excited directly (without utilisation of the donor). However, if the acceptor is *non-fluorescent*, it will merely accept excitation energy from the donor, yet not produce any fluorescence by its own. Such acceptors are called "**fluorescence quenchers**".

Fluorescence quenchers reduce the fluorescence intensity of the donor dye according to the formulas given on p. 10-11. The Förster-radius $R_0$ is determined by the overlap between fluorescence spectrum of the donor and absorption spectrum of the acceptor (quencher). For efficient quenching the absorption region of the quencher must overlap well with the fluorescence spectrum of the donor.

**ATTO-TEC** provides quenchers covering most of the relevant visible spectrum. Their properties are outlined on p. 61-63. The Förster-radii $R_0$ for combinations with fluorescent ATTO-labels as donors are presented in the table on p. 12-13.

*Note:*

The fluorescence of dyes may be quenched also by mechanisms entirely different than FRET. For example, the fluorescence of **ATTO 655**, **ATTO 680**, and **ATTO 700** is quenched very efficiently by guanosine, tryptophan and related compounds. This process is based on electron transfer and requires direct contact between excited dye molecule and quenching agent.



Fluorescence Quenchers

## ATTO 540Q

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 542 nm

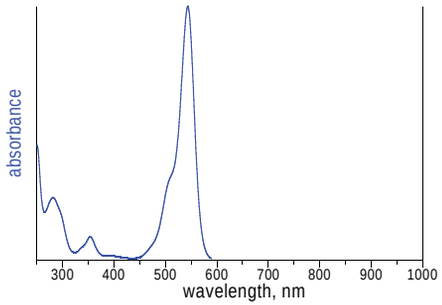
$\varepsilon_{max}$ = 1.05 x $10^5$ $M^{-1}$ $cm^{-1}$

$CF_{260}$ = 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 Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 659 | 559 | AD 540Q-21 | AD 540Q-25 |
| NHS-ester | 756 | 656 | AD 540Q-31 | AD 540Q-35 |
| maleimide | 781 | 681 | AD 540Q-41 | AD 540Q-45 |
| streptavidin | | | AD 540Q-61 | AD 540Q-65 |
| biotin | 969 | 869 | AD 540Q-71 | AD 540Q-75 |

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

## ATTO 580Q

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 586 nm

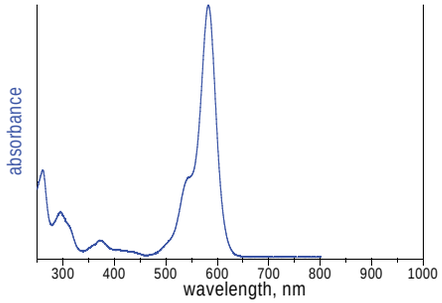
$\varepsilon_{max}$ = 1.1 x $10^5$ $M^{-1}$ $cm^{-1}$

$CF_{260}$ = 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 Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 795 | 695 | AD 580Q-21 | AD 580Q-25 |
| NHS-ester | 892 | 792 | AD 580Q-31 | AD 580Q-35 |
| maleimide | 917 | 817 | AD 580Q-41 | AD 580Q-45 |
| biotin | 1105 | 1005 | AD 580Q-71 | AD 580Q-75 |

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





## ATTO 612Q

**Optical properties of carboxy derivative**

$\lambda_{abs}$ = 615 nm

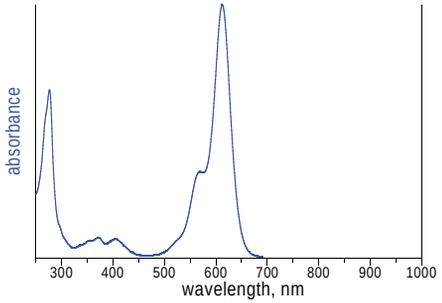
$\varepsilon_{max}$ = 1.15 x $10^5$ $M^{-1}$ $cm^{-1}$

$CF_{260}$ = 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 Unit (1 mg) | Order Code Unit (5 mg) |
|---|---|---|---|---|
| with free COOH | 791 | 691 | AD 612Q-21 | AD 612Q-25 |
| NHS-ester | 888 | 788 | AD 612Q-31 | AD 612Q-35 |
| maleimide | 913 | 813 | AD 612Q-41 | AD 612Q-45 |
| streptavidin | | | AD 612Q-61 | AD 612Q-65 |
| biotin | 1101 | 1001 | AD 612Q-71 | AD 612Q-75 |

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

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.

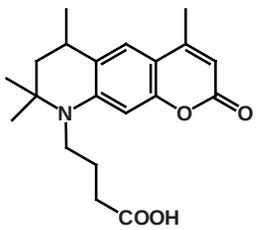
On excitation of dyes with highly *unsymmetrical* π-electron systems the dipole moment may change drastically. The ensuing strong reorientation of solvent molecules leads to an unusually large Stokes-shift, in particular in polar solvents like water and ethanol. As the non-radiative decay of the excited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

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

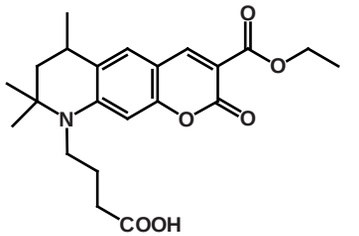
**Optical Properties in Water**

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

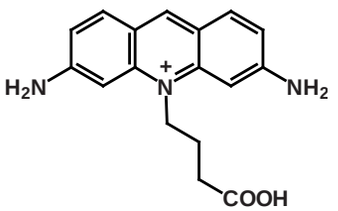
**ATTO 390**



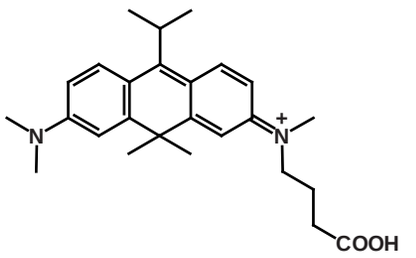

**ATTO 425**




**ATTO 465**




**ATTO 611X**



## Customized Labels and Products

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

## Derivatives of ATTO-Labels

### *Linker*

In most ATTO-labels the reactive group (NHS-ester etc.) is connected with the fluorophore by a linker consisting of a 4-atom flexible chain. For many applications this has proven to be very suitable and practical. However, if your experiment requires a linker of different length, rigidity, or other special feature, - most likely we are able to provide it.

### *Reactive Group*

N-hydroxysuccinimide (NHS) ester and maleimide are the most common reactive groups for coupling to amine and thiol, respectively. However, for other substrate functionalities it is necessary that the label carries an entirely different reactive group: ATTO-TEC can provide amino-, hydrazine-groups, phosphoramidites, products for “Click-Chemistry“ (azides, alkynes) and many others.

### *Solubility, Charges*

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



Customized Dyes and Services

### *Conjugates*

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

## Special Dyes

### *Bichromophoric Dyes*

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

### *pH-Sensitive Dyes*

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

### *Protease-Active Dyes*

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

Labeling Procedures

## Recommended Procedures for Labeling

***Introduction***

**ATTO-TEC** offers a large variety of high-quality dyes for labeling amino and thiol groups. ATTO reactive dyes cover the spectral region from 350 nm in the UV to 750 nm in the NIR. The most commonly used amine-reactive reagents are N-hydroxysuccinimidyl(NHS)-esters. NHS-esters readily react with amine-modified oligonucleotides or amino groups of proteins, i.e. the ε-amino groups of lysines or the amine terminus, forming a chemically stable amide bond between the dye and the protein or oligo. However, the amino group ought to be unprotonated to be reactive. Therefore the pH of the solution must be adjusted sufficiently high to obtain a high concentration of unprotonated amino groups. On the other hand, the NHS-ester also reacts with the hydroxyl ions in the solution to yield free dye, which is no longer reactive. As the rate of this hydrolysis increases with the concentration of hydroxyl ions, the pH should be kept as low as possible. Buffering the solution at pH 8.3 has been found to be a good compromise between the contradicting requirements. Isothiocyanates also react with amino groups. However, in general the resulting thiourea compound is less stable and deteriorates over time. Sulfonyl chlorides are another group of amine-reactive compounds forming very stable sulfonamides, yet are more difficult to work with. Therefore NHS-esters are the preferred amine-reactive reagents for protein- or oligo-conjugation. For the labeling of thiol groups the most popular and commonly used reactive reagents are maleimides. ATTO maleimides react with thiol groups of proteins to form a stable thio-ether bond. Unlike the labeling of amino groups, thiol modifications generally take place at near neutral pH. Since most amino groups show very little reactivity at pH 7, thiol groups can be selectively labeled in the presence of amines.

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

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

***Procedure***

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

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

***Separation of the Conjugate from Free Dye***

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

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

***Storage of the Protein Conjugate***

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

***Determining the Degree of Labeling (DOL)***

The degree of labeling (DOL, dye-to-protein ratio) obtained by the above procedure can be determined by absorption spectroscopy making use of the Lambert-Beer law: Absorbance (A) = extinction coefficient (ε) × molar concentration × path length (d). Simply measure the UV-VIS spectrum of the conjugate solution as obtained after gel filtration in a quartz (UV-transparent) cell. You may need to dilute the solution, if it turns out to be too concentrated for a correct absorbance measurement. Determine the absorbance ($A_{max}$) at the absorption maximum ($\lambda_{abs}$) 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) = $A_{max}$ / $\varepsilon_{max}$ × d, where $\varepsilon_{max}$ is the extinction coefficient of the dye at the absorption maximum. The protein concentration is obtained in the same way from its absorbance at 280 nm. As all dyes show some absorption at 280 nm, the measured absorbance $A_{280}$ must be corrected for the contribution of the dye. This is given by $A_{max}$ × $CF_{280}$. The values for the correction factor $CF_{280}$ = $\varepsilon_{280}$ / $\varepsilon_{max}$ are listed in the table on p.75. 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) = $A_{prot}$ / $\varepsilon_{prot}$ × d, where $\varepsilon_{prot}$ is the extinction coefficient of the protein at 280 nm.



Labeling Procedures

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

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

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

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

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

***Procedure***

Dissolve the protein at 50 - 100 µM in PBS at pH 7.0 - 7.5. Reduction of disulfide bonds in the protein can be achieved by adding a tenfold molar excess of dithiothreitol (DTT) or other reducing agent. If DTT is used as a reducing agent, the excess has to be removed by dialysis prior to addition of the maleimide. This may not be necessary with other reducing agents. We recommend to carry out the thiol modification in an inert atmosphere to prevent oxidation of the thiols. It may also be advisable to deoxygenate all buffers and solvents used for the thiol conjugation.
Prepare a 1 - 10 mM stock solution of the ATTO maleimide in anhydrous DMSO or DMF. Note that such solutions are not stable for a long period of time. Hence we recommend to freshly prepare the dye solutions immediately prior to use. Add a 10 - 20 fold molar excess of reactive dye to the protein solution whilst stirring and incubate 2 hours at room temperature.

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

***Separation of the Conjugate from Free Dye***

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

## Labeling Amine-Modified Oligonucleotides

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

***Purification of the Amine-Modified Oligonucleotide***

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



Labeling Procedures

***Recommended Buffers***

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

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

***Procedure***

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

***Separation and Purification of the Conjugate***

We recommend the precipitation of the oligonucleotide with ethanol as the first step of purification. To achieve this, add 10 µl of 3 M sodium chloride and 250 µl of ethanol to the reaction mixture. Proceed as described under *Purification of the amine-modified oligonucleotide.*
Note: Do not dry completely as the labeled oligonucleotide may become difficult to redissolve. The labeled oligonucleotide can be separated from unlabeled oligonucleotide and free dye by preparative gel electrophoresis or reversed-phase HPLC.

### *Purification by Gel Electrophoresis*

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

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

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

### *Purification by HPLC*

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



Labeling Procedures

**Table: Optical properties of ATTO-labels**

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

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

*Features:*

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

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

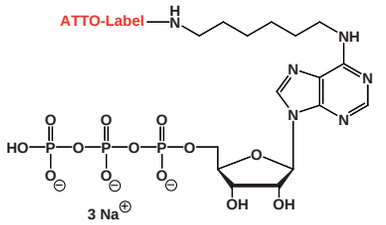
To create the applicable order code, please replace the xxx in the order codes below by the **ATTO**-dye number.

## Labeled Adenosine Nucleotides

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

Order code:

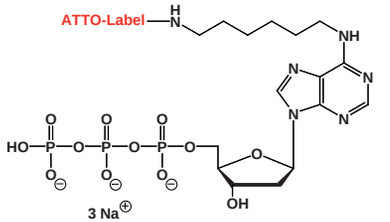
NU-805-xxx




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

Order code:

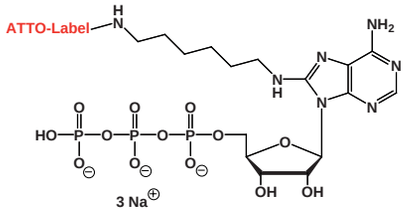
NU-835-xxx




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

Order code:

NU-807-xxx



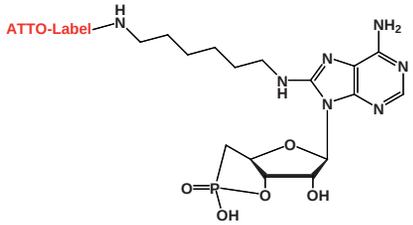





### 8-[(6-Amino)hexyl]-amino-cAMP

Order code:

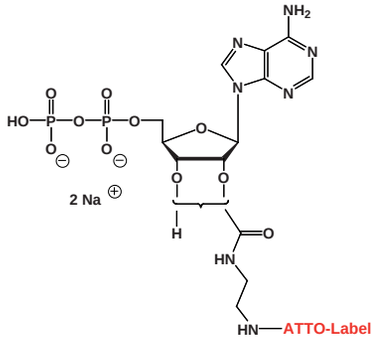
NU-851-xxx




### EDA-ADP

Order code:

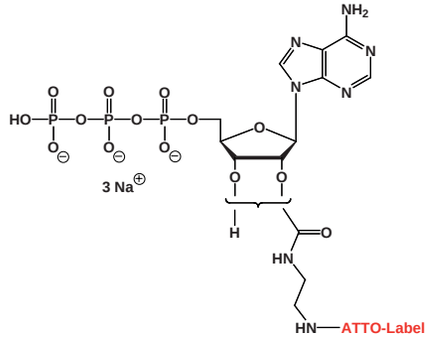
NU-802-xxx




### EDA-ATP

Order code:

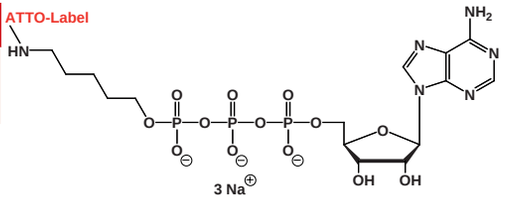
NU-808-xxx




### γ-(6-Aminohexyl)-ATP

Order code:

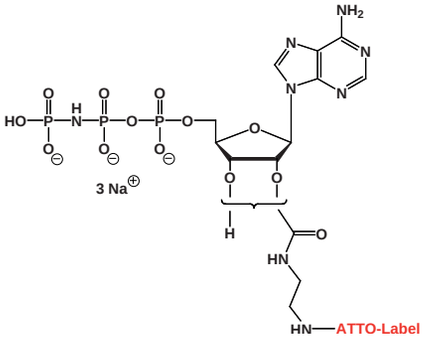
NU-833-xxx



## EDA-AppNHp (EDA-AMPPNP)

Order code:

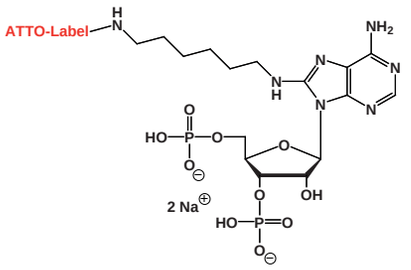
NU-810-xxx




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

Order code:

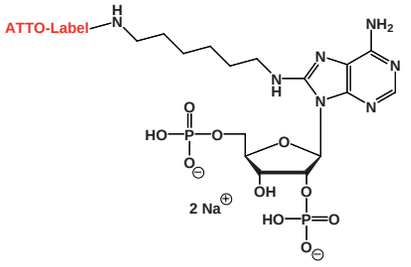
NU-811-xxx




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

Order code:

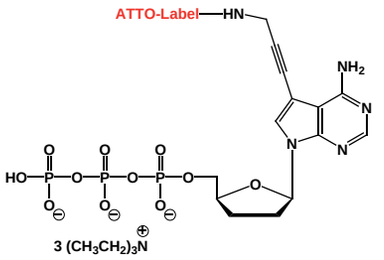
NU-812-xxx




## 7-Propargylamino-7-deaza-ddATP

Order code:

NU-1612-xxx



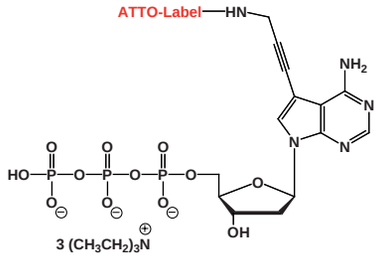



Labeled Nucleotides

## 7-Propargylamino-7-deaza-dATP

Order code:

NU-1611-xxx

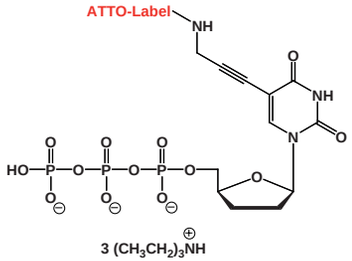



### Labeled Cytidine Nucleotides

## 5-Propargylamino-ddCTP

Order code:

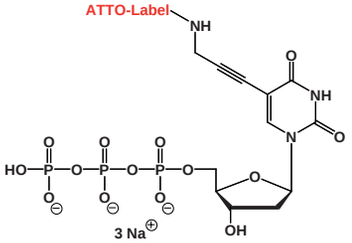
NU-850-xxx




## 5-Propargylamino-dCTP

Order code:

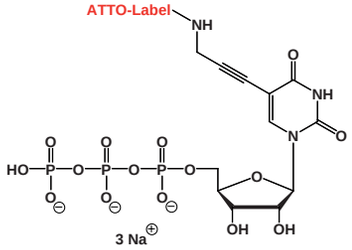
NU-809-xxx




## 5-Propargylamino-CTP

Order code:

NU-831-xxx



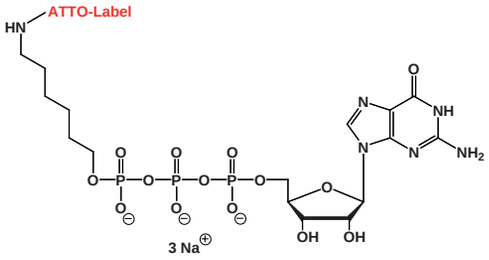
Labeled Nucleotides

## Labeled Guanosine and m$^7$ Guanosine Nucleotides

### γ-(6-Aminohexyl)-GTP

Order code:

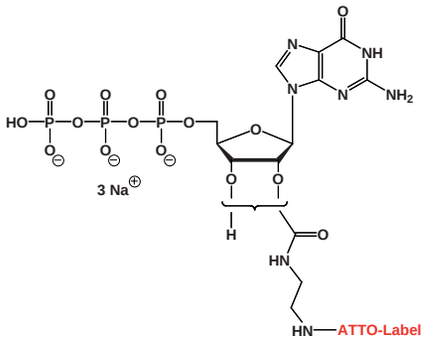
NU-834-xxx




### EDA-GTP

Order code:

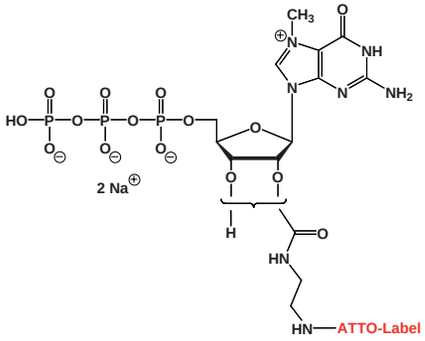
NU-820-xxx




### EDA-m$^7$-GTP

Order code:

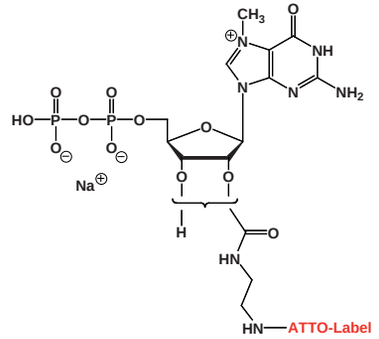
NU-824-xxx




### EDA-m$^7$-GDP

Order code:

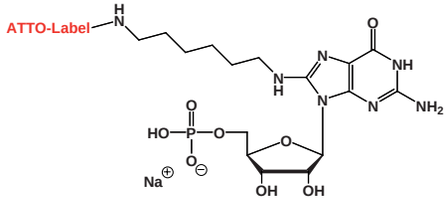
NU-827-xxx




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

Order code:

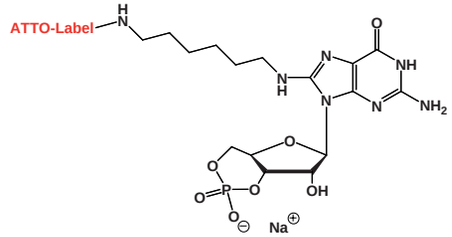
NU-829-xxx




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

Order code:

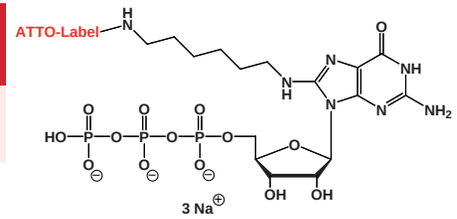
NU-832-xxx




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

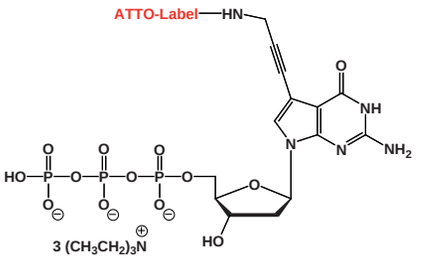
Order code:

NU-830-xxx



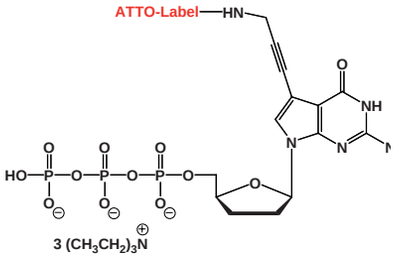
## 7-Propargylamino-7-deaza-dGTP

Order code:
NU-1615-xxx




## 7-Propargylamino-7-deaza-ddGTP

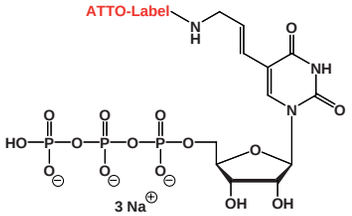
Order code:
NU-1618-xxx




## Labeled Uridine Nucleotides

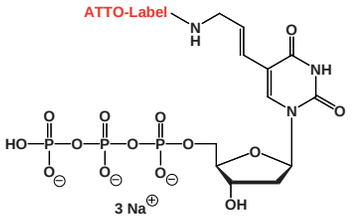
## Aminoallyl-UTP

Order code:
NU-821-xxx




## Aminoallyl-dUTP

Order code:
NU-803-xxx








## 5-Propargylamino-ddUTP

Order code:
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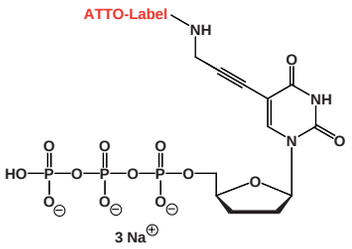
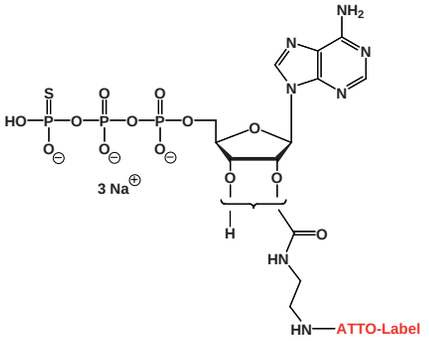



## Labeled ATPγS and GTPγS Nucleotides

## EDA-ATPγS

Order code:
NU-1609-xxx




## EDA-GTPγS

Order code:
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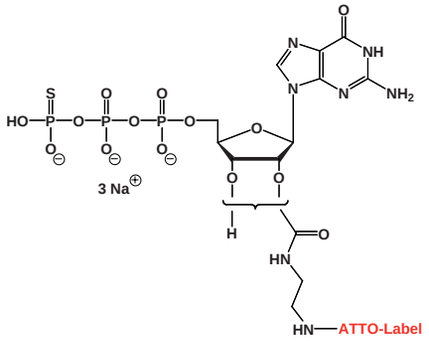
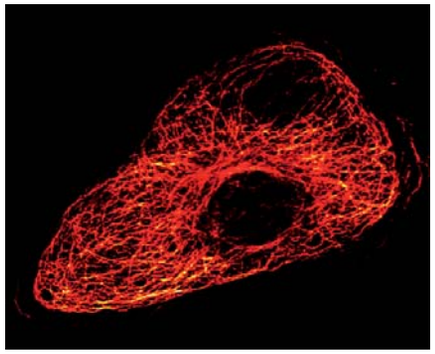
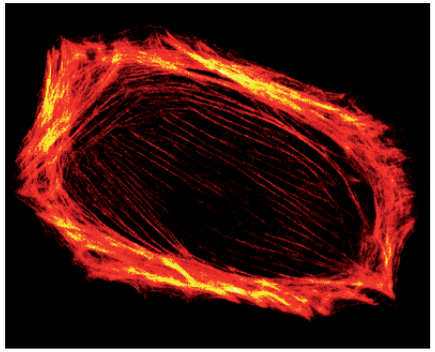



**ATTO-Labels**:

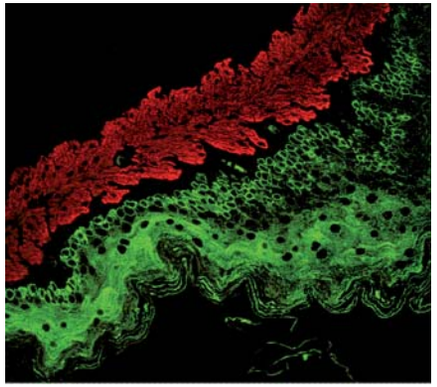
| | | | | |
|---|---|---|---|---|
| ATTO 390 | ATTO 425 | ATTO 465 | ATTO 488 | ATTO 495 |
| ATTO 532 | ATTO Rho 6G | ATTO 540Q | ATTO 550 | ATTO 565 |
| ATTO Rho3B | ATTO Rho11 | ATTO Rho12 | ATTO Thio12 | ATTO 580Q |
| ATTO Rho101 | ATTO 590 | ATTO 594 | ATTO Rho13 | ATTO 612Q |
| ATTO 620 | ATTO Rho14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



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



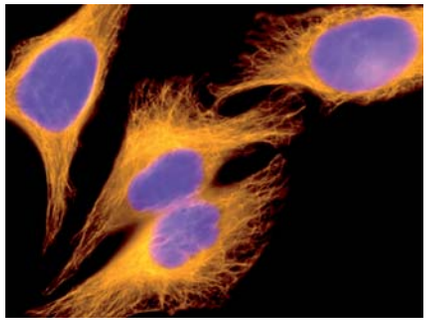
*Actin* (PtK2 - Male Rat Kangaroo Kidney Epithelial Cells)
**ATTO 532** labeled phalloidin bound to actin.



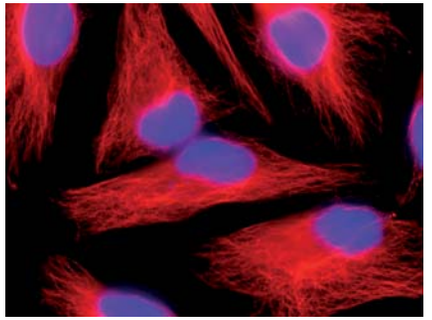
Rat stomach: Actin stained with mouse anti-smooth muscle $\alpha$-actin antibody and **ATTO 488** anti-mouse IgG (green). Cytokeratin stained with polyclonal rabbit anti-cytokeratin and **ATTO 647N** anti-rabbit IgG (red).



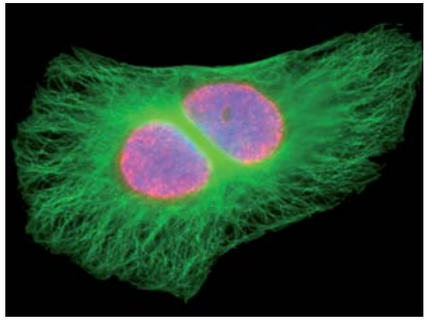




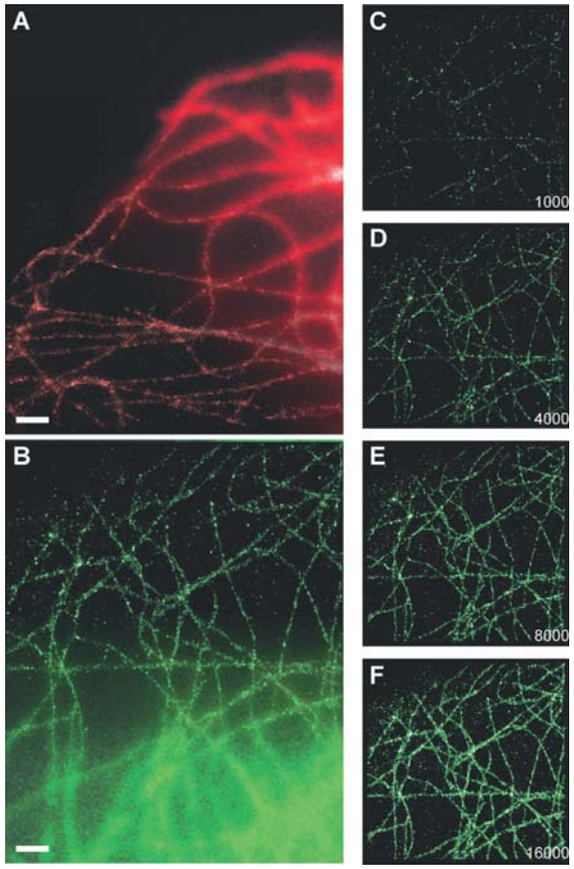
HeLa cells stained with LP Bio anti-alpha Tubulin, clone 5-B-1-2 primary and the Vendikar Yellow **ATTO 550** anti-mouse secondary. Blue: DAPI staining. Widefield image acquired with Zeiss Axio Observer.Z1 with halogen illumination.



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



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

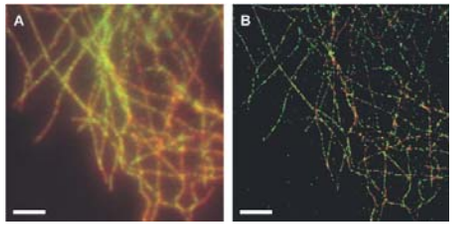



Photoswitching microscopy performed with the two standard fluorophores **ATTO 655** and **ATTO 520**. The upper right (A) and lower (B) parts of the images are conventional immuno-fluorescence images of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** (A) and **ATTO 520**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

M. Sauer et al., Bielefeld University.

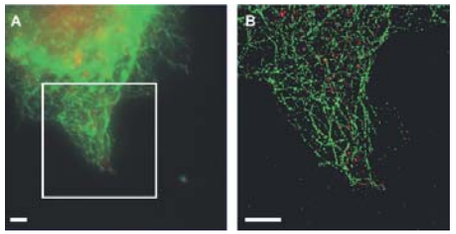




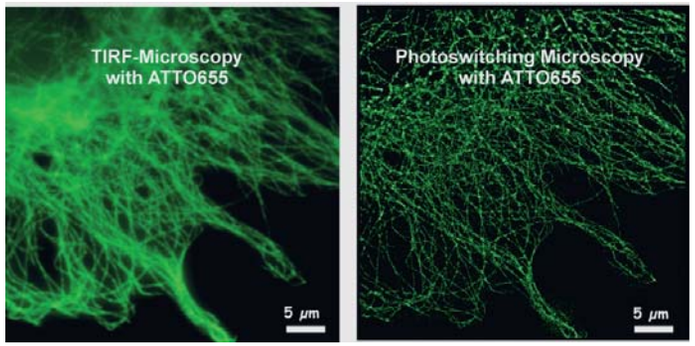



Dual-color photoswitching microscopy with **ATTO 520** and **ATTO 655** labeled microtubules in COS-7 cells. The reconstructed photoswitching image is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed sequentially in PBS, pH 7.4 in the presence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

M. Sauer et al., Bielefeld University.

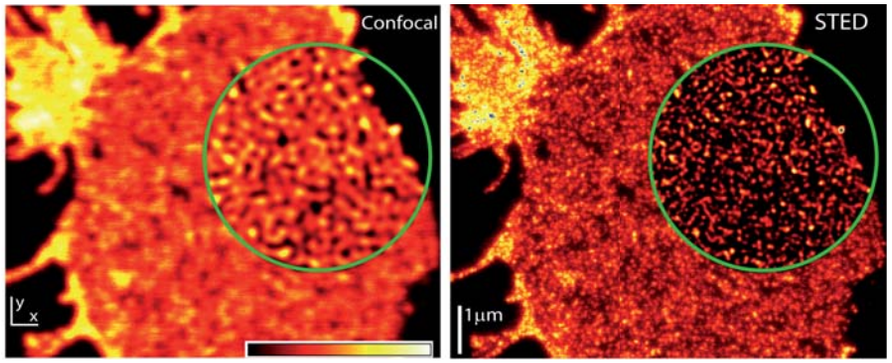



Dual-color photoswitching microscopy with **ATTO 520** labeled microtubules and **ATTO 655** labeled cytochrome c oxidase localized in the inner mitochondrial membrane of COS-7 cells. The reconstructed dual-color photoswitching image (expanded section) is shown in (B) and compared to the corresponding conventional wide-field image (A). Measurements were performed successively in PBS, pH7.4 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).



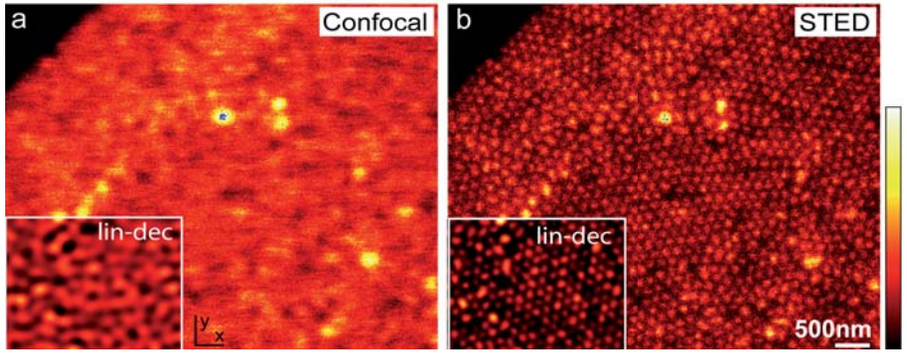

Photoswitching microscopy performed with **ATTO 655**. The left side shows a conventional immuno-fluorescence image of microtubules in COS-7 cells labeled with a primary antibody and **ATTO 655** labeled F(ab')2 fragments. The right side shows photoswitching microscopy image with subdiffraction resolution (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., Bielefeld University.




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

S.W. Hell et al., MPI Göttingen.

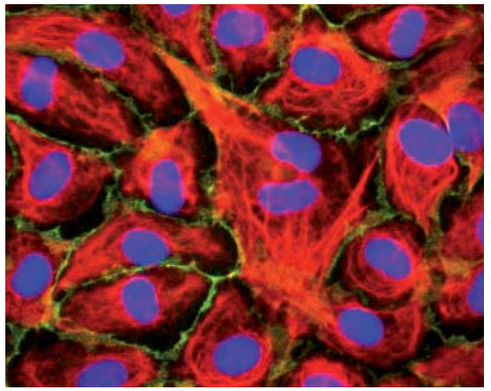



Revealing the nanopattern of the SNARE protein SNAP-25 on the plasma membrane of a mammalian cell. Confocal versus STED image of the antibody-tagged proteins. The secondary antibody was labeled with **ATTO 532-NHS**. The encircled areas show linearly deconvolved data. STED microscopy provides a substantial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

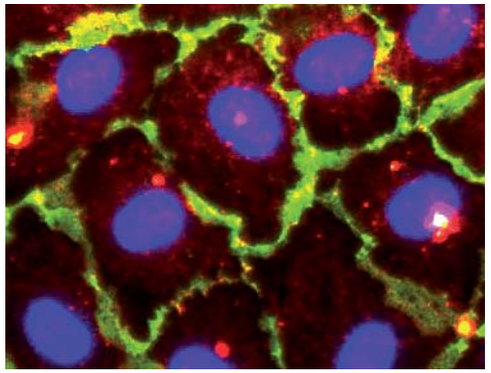
S.W. Hell et al., MPI Göttingen.



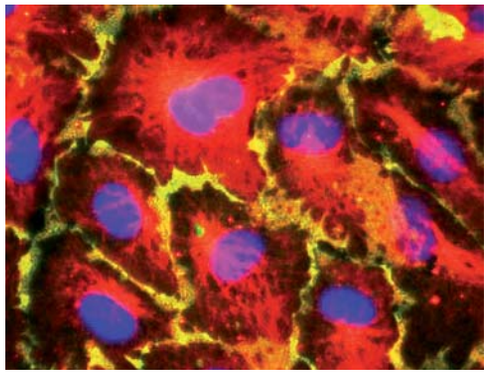




HUVEC: Vimentin/**ATTO 532**; E-Cadherin/**ATTO 655** and DAPI



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



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

| Abbreviation | |
|---|---|
| $\lambda$ | wavelength |
| $\lambda_{abs}$ | longest-wavelength absorption maximum |
| $\varepsilon_{max}$ | molar extinction coefficient at the longest-wavelength absorption maximum |
| $\varepsilon_{260}$ | molar extinction coefficient at $\lambda$ = 260 nm |
| $\varepsilon_{280}$ | molar extinction coefficient at $\lambda$ = 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. |
| $\lambda_{fl}$ | fluorescence maximum |
| $\eta_{fl}$ | fluorescence quantum yield |
| $\tau_{fl}$ | fluorescence decay time |
| $\tau_0$ | natural (radiative) decay time |
| MW | molecular weight |
| $M^+$ | molecular weight of dye cation (HPLC-MS) |
| $MH^+$ | molecular weight of protonated dye (HPLC-MS) |
| PBS | phosphate buffered saline |
| DOL | degree of labeling |
| HUVEC | human umbilical vein endothelial cells |
| DAPI | 4‘,6-diamidino-2-phenylindole |
| FITC | fluorescein isothiocyanate |
| TAMRA | 6-carboxytetramethylrhodamine |
| FAM | 6-carboxyfluorescein |
| TET | tetrachloro-6-carboxyfluorescein |
| JOE | 2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein |
| HEX | hexachloro-6-carboxyfluorescein |
| ROX | 6-carboxy-X-rhodamine |
| | |





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

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

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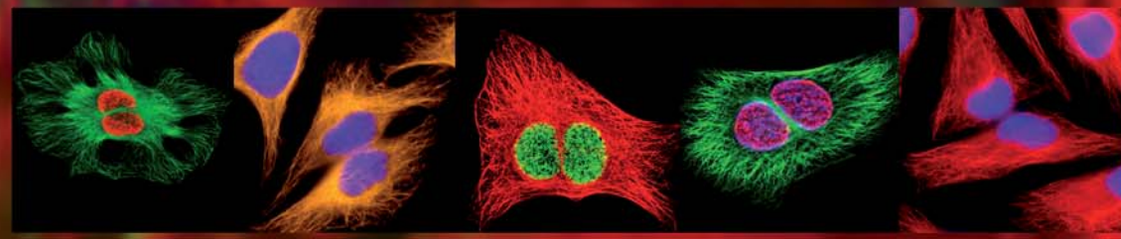

# *Fluorescent Labels and Dyes*

## *catalogue 2009/2010*