

ATTO-TEC GmbH

Martinshardt 7

D-57074 Siegen

Germany

Phone: +49 271 23853 – 0

FAX: +49 271 23853 – 11

E-mail: info@atto-tec.com

http: www.atto-tec.com



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Product Information: ATTO 514

ATTO 514 is a new hydrophilic fluorescent label with excellent water solubility. The dye exhibits strong absorption, high fluorescence quantum yield and exceptional thermal and photo-stability. Thus **ATTO 514** is highly suitable for single-molecule detection applications and high-resolution microscopy such as PALM, dSTORM, STED etc. Additionally the dye highly qualifies to be applied in flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more. The fluorescence is excited most efficiently in the range 510 - 535 nm. A suitable source of excitation is the 514 nm line of the Argon-Ion laser. For details of coupling see our recommended labeling procedures at www.atto-tec.com - Support - [User Guides & Protocols](#).

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Optical data of the carboxy derivative (in PBS, pH 7.4):

$$\lambda_{\text{abs}} = 511 \text{ nm}$$

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

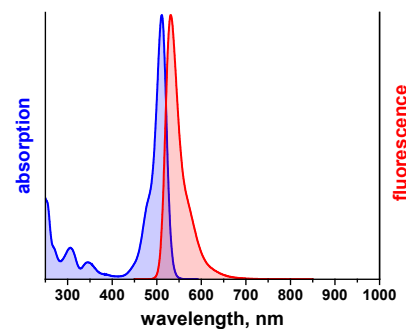
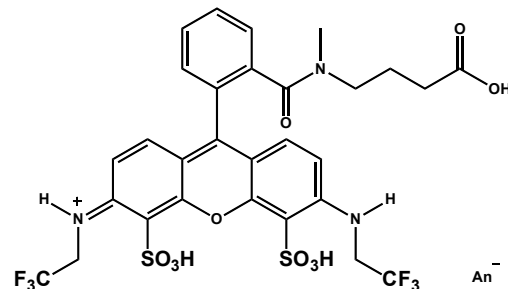
$$\lambda_{\text{fl}} = 532 \text{ nm}$$

$$\eta_{\text{fl}} = 85 \%$$

$$\tau_{\text{fl}} = 3.9 \text{ ns}$$

$$\text{CF}_{260} = 0.21$$

$$\text{CF}_{280} = 0.07$$



Spectra available in digitized form (excel file) on <http://www.atto-tec.com>

Modification	MW, g/mol	M ⁺ , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
carboxy	868	754	AD 514-21	AD 514-25
NHS-ester	1111	851	AD 514-31	AD 514-35
maleimide	990	876	AD 514-41	AD 514-45
biotin	1178	1064	AD 514-71	AD 514-75
phalloidin	1638	1523	AD 514-81*	AD 514-82**
amine	1024	796	AD 514-91	AD 514-95
azide	1068	954	AD 514-101	AD 514-105
iodoacetamide	1078	964	AD 514-111	AD 514-115
hydrazide <i>new</i>	882	768	AD 514-121	AD 514-125
alkyne	905	791	AD 514-141	AD 514-145

* 10 nmol **20 nmol

General Information

Storage: The product is shipped solvent-free at ambient temperature. Upon receipt store at -20 °C. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening. When stored properly, protected from moisture and light, ATTO-TEC products are stable for at least three years.

Risk and safety: A material safety data sheet (MSDS) of each derivative can be downloaded from our website at www.atto-tec.com.

Solutions: The product is soluble in polar solvents, e.g. dimethylformamide (DMF), dimethylsulfoxide (DMSO). However, due to their inherent reactivity, NHS-esters and maleimides must be well protected from OH-containing solvents like ethanol and, in particular, water. Prepare labeling solutions of NHS-esters and maleimides immediately before use by dissolving the vial content in anhydrous and amine-free DMF or DMSO. Depending on the quality of the solvent used, such solutions may be of limited stability.

Dye with **free carboxy group (COOH)** may be used for any kind of spectroscopy. Stock solutions can be prepared with water or aqueous buffer. Due to the high extinction coefficient and its high quantum yield of fluorescence this product is suitable for high-sensitivity detection including single-molecule work. The dye can be activated at the carboxy group for coupling purposes.

The **NHS-ester** of the dye reacts easily with amino-groups of proteins and other bio-molecules. Since the amino-group must be non-protonated to be reactive, the pH of the reaction solution has to be adjusted sufficiently high. As with all NHS-esters unavoidable hydrolysis takes place at high pH and competes with the desired labeling reaction. Therefore the solution has to be buffered carefully. For details see the Labeling Protocol on www.atto-tec.com.

The **maleimide** is suitable for labeling sulfhydryl (thiol) groups of proteins, in particular cystein residues. See Labeling Protocol on www.atto-tec.com.

The **biotin** derivative can be used as reagent for binding to proteins like avidin and streptavidin.

Phalloidin, a bicyclic heptapeptide, is a very strong binding reagent to actin. Fluorescent labeled phalloidin has become a useful tool to investigate the distribution of F-actin within the cytoskeleton of cells by fluorescence microscopy. To prepare a stock solution of the phalloidin-conjugate it is recommended dissolving the sample in 1 ml of methanol.

The **amine** derivative may be used for reactions with activated carboxy-groups like NHS-esters, TFP-esters etc.

The **hydrazide** derivative is used to modify aldehydes and ketones.

The **azide** or **alkyne** modification is used in the Huisgen reaction ("Click Chemistry").

The **iodoacetamide** derivative reacts, like the maleimide, with a sulfhydryl group forming a thioether bond. It is predominantly used for tagging cystein residues of proteins.

Further Notes:

- ATTO-TEC products are high-quality reagents intended for research purposes only.
- The use of ATTO-TEC products must be supervised by technically qualified personnel experienced in handling potentially hazardous chemicals. For safety instructions please read the corresponding Material Safety Data Sheet.
- Most ATTO-TEC products and product applications are covered by European and foreign patents.
- Commercial use of ATTO-TEC products is not permitted without written agreement by ATTO-TEC GmbH. Inquiries for licensing may be directed to info@atto-tec.com.