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Product Information: ATTO 565

ATTO 565 is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is intended for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy.

As supplied **ATTO 565** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer. For details of coupling see our recommended labeling procedure at www.atto-tec.com - Support - [User Guides & Protocols](#).

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

$$\lambda_{\text{abs}} = 564 \text{ nm}$$

$$\epsilon_{\text{max}} = 1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

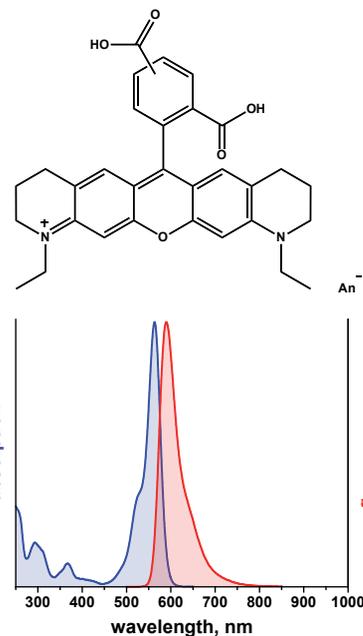
$$\lambda_{\text{fl}} = 590 \text{ nm}$$

$$\eta_{\text{fl}} = 90 \%$$

$$\tau_{\text{fl}} = 4.0 \text{ ns}$$

$$\text{CF}_{260} = 0.27$$

$$\text{CF}_{280} = 0.12$$



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	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
biotin	922	821	AD 565-71	AD 565-75
phalloidin	1394	1280	AD 565-81*	AD 565-82**
amine	781	553	AD 565-91	AD 565-95
Peg(3)-azide	811	711	AD 565-101	AD 565-105
iodoacetamide	835	721	AD 565-111	AD 565-115
hydrazide	752	525	AD 565-121	AD 565-125
alkyne	648	548	AD 565-141	AD 565-145
cadaverine	796	596	AD 565-231	AD 565-235
tetrazine (MeTet)	794	694	AD 565-2502 [#]	AD 565-2505 ^{##}
Peg(4)-DBCO <i>new</i>	1016	1016	AD 565-291	AD 565-295

* 10 nmol **20 nmol #0.2 mg ##0.5 mg

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), or acetonitrile. 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. 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 **azide** or **alkyne** and **DBCO** modification are “click-reagents“ and used in the Huisgen reaction and in case of DBCO in a “strain-promoted“ azide-alkyne cycloaddition (“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.

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

The **cadaverine** derivative can be used as a fluorescent amine donor substrate for transglutaminases.

The **tetrazine** derivative readily reacts in a bioorthogonal way with strained alkenes or alkynes such as trans-cyclooctenes (TCO) or cyclooctynes like bicyclo[6.1.0]non-4-yne (BCN), respectively.

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.