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Product Information: ATTO 532

ATTO 532 is a fluorescent label related to the well-known dye Rhodamine 6G. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, excellent water solubility. Thus **ATTO 532** is highly suitable for single-molecule detection applications and high-resolution microscopy such as PALM, dSTORM, SIM, 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 515 - 545 nm. A suitable excitation source for **ATTO 532** is the 532 nm output of the frequency-doubled Nd:YAG laser. 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):

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

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

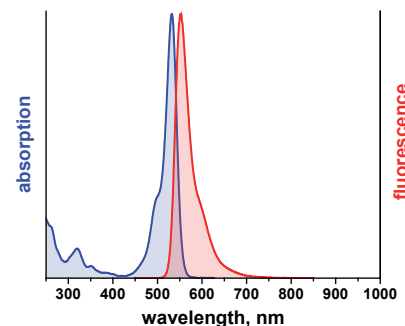
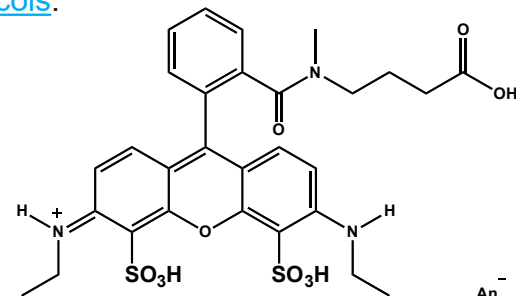
$$\lambda_{\text{fl}} = 552 \text{ nm}$$

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

$$\tau_{\text{fl}} = 3.8 \text{ ns}$$

$$\text{CF}_{260} = 0.20$$

$$\text{CF}_{280} = 0.09$$



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	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
biotin	1357	956	AD 532-71	AD 532-75
phalloidin	1530	1415	AD 532-81*	AD 532-82**
amine	916	688	AD 532-91	AD 532-95
Peg(3)-azide	884	846	AD 532-101	AD 532-105
iodoacetamide	970	856	AD 532-111	AD 532-115
hydrazide	774	660	AD 532-121	AD 532-125
alkyne	797	683	AD 532-141	AD 532-145
cadaverine	958	730	AD 532-231	AD 532-235
tetrazine (MeTet)	943	829	AD 532-2502 [#]	AD 532-2505 ^{##}
Peg(4)-DBCO <i>new</i>	1189	1152	AD 532-291	AD 532-295

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

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 **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.