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Revised: 2024-02-09

Product Information: **ATTO 643**



ATTO 643 is a new fluorescent label related to the well known dye ATTO 647N. The new label is very **hydrophilic**, hence it shows excellent water solubility and very little tendency for unspecific binding. Characteristic features of the dye are strong absorption, high photostability, and very little triplet formation. **ATTO 643** exhibits an extraordinary high fluorescence quantum yield, which is only slightly reduced after conjugation to biomolecules, e.g. antibodies, streptavidin..., even at high degrees of labeling (DOL).

The dye is very suitable for single-molecule detection applications and high-resolution microscopy (SIM, STED, etc.). The label is also highly recommended for oligonucleotide labeling, flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more.

ATTO 643 is an anionic dye (charge -1). As supplied the dye consists of three isomers with practically identical absorption and fluorescence. 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):

$$\lambda_{\text{abs}} = 643 \text{ nm}$$

$$\epsilon_{\text{max}} = 1.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

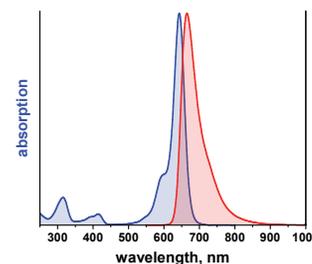
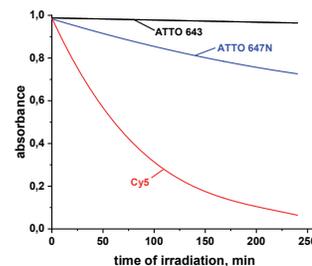
$$\lambda_{\text{fl}} = 665 \text{ nm}$$

$$\eta_{\text{fl}} = 62 \%$$

$$\tau_{\text{fl}} = 3.5 \text{ ns}$$

$$\text{CF}_{260} = 0.05$$

$$\text{CF}_{280} = 0.04$$



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	950	836	AD 643-21	AD 643-25
NHS-ester	955	933	AD 643-31	AD 643-35
maleimide	1072	958	AD 643-41	AD 643-45
biotin	1261	1147	AD 643-71	AD 643-75
phalloidin	1741	1627	AD 643-81*	AD 643-82**
amine	1106	878	AD 643-91	AD 643-95
Peg(3)-azide	1058	1036	AD 643-101	AD 643-105
alkyne	987	873	AD 643-141	AD 643-145
tetrazine (MeTet)	1019	1019	AD 643-2502 [#]	AD 643-2505 ^{##}
Peg(4)-DBCO <i>new</i>	1364	1343	AD 643-291	AD 643-295

* 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 **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.