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**Product Information: ATTO 655**

**ATTO 655** belongs to a new generation of fluorescent labels. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, good fluorescence quantum yield, excellent thermal and photo-stability, outstanding ozone resistance, very good water solubility, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy PALM, dSTORM, SIM, STED etc.

**ATTO 655** is a zwitterionic dye with a net electrical charge of zero. The fluorescence is efficiently quenched by electron donors like guanine, tryptophan, etc. For details of coupling see our recommended labeling procedure at [www.atto-tec.com](http://www.atto-tec.com) - Support - Downloads - [General Procedures](#).

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

$$\lambda_{\text{abs}} = 663 \text{ nm}$$

$$\epsilon_{\text{max}} = 1.25 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$$

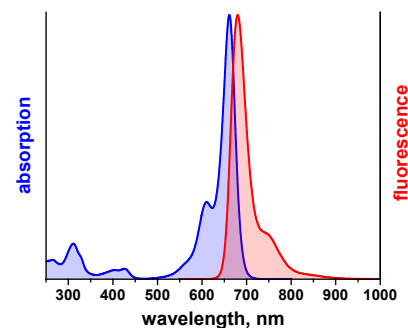
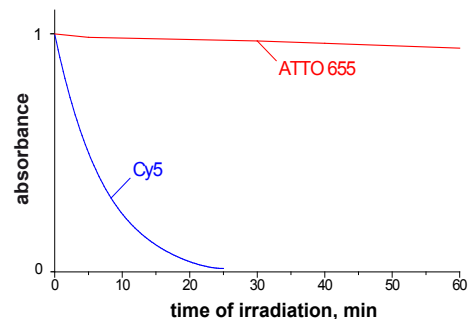
$$\lambda_{\text{fl}} = 680 \text{ nm}$$

$$\eta_{\text{fl}} = 30 \%$$

$$\tau_{\text{fl}} = 1.8 \text{ ns}$$

$$\text{CF}_{260} = 0.24$$

$$\text{CF}_{280} = 0.08$$



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

Modification	MW, g/mol	M <sup>+</sup> , g/mol	Order Code	
			Unit (1 mg)	Unit (5 mg)
carboxy	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
biotin	1204	838	AD 655-71	AD 655-75
phalloidin	1412	1297	AD 655-81*	AD 655-82**
amine	798	570	AD 655-91	AD 655-95
azide	842	728	AD 655-101	AD 655-105
iodoacetamide	852	738	AD 655-111	AD 655-115
alkyne	679	565	AD 655-141	AD 655-141
tetrazine (MeTet)	825	711	AD 655-2502#	AD 655-2505##

\*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](http://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](http://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](http://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** 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.

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.
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