

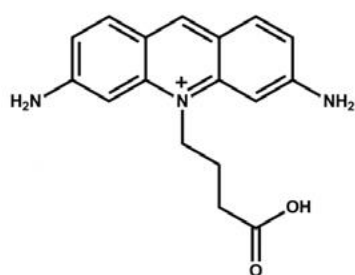
# 53404 Atto 465 NHS ester

## Application

Atto 465 is a novel fluorescent label derived from the well-known dye Acridavine.

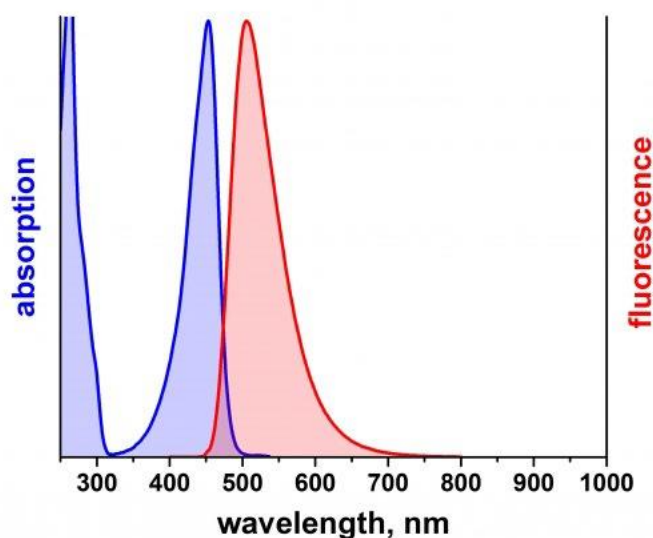
The dye is intended for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption and good fluorescence, large Stokes-shift, good water solubility and high triplet quantum yield. The dye shows intense and long-lived phosphorescence in solid matrix.

## Product Description



MW	493 g/mol
$\lambda_{\text{abs}}$	453 nm
$E_{\text{max}}$	$7.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
$\lambda_{\text{fl}}$	506 nm
$\eta_{\text{fl}}$	75 %
$T_{\text{fl}}$	5.0 ns
CF <sub>260</sub>	1.09
CF <sub>280</sub>	0.48

### Optical data of the carboxy derivative (in aqueous solution)



## Storage

protected from moisture and light at -20°C

## Directions for labelling of proteins with Atto 465 NHS ester

- Dissolve protein in bicarbonate buffer (0.1 M, preferably of pH 8.3) at 2 mg/ml. Concentrations below 2 mg protein per ml will decrease labeling efficiency. Protein or peptide solutions must be free of any amine-containing substances such as Tris, glycine or ammonium salts. Antibodies in solutions of Tris buffers may be dialyzed against 10-20 mM PBS. The desired pH can be obtained by adding 0.1 ml of 1 M sodium bicarbonate buffer, pH 8.3, for each ml of dialyzed antibody solution.
- Dissolve Atto 465 NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto 465 NHS in 500 µl). This solution should always be prepared immediately before conjugation.



As number and position of amine groups vary between different proteins, the optimum of dye/protein ratio also varies. Wherefore we recommend trying out different ratios when labeling a certain protein for the first time.

- In general, a ratio of 1-2 may be suitable. To obtain a ratio in this range, add a twofold molar excess of reactive dye to the protein solution. For an antibody, add 10 µl of dye solution to 1 ml protein solution.
- Incubate the reaction at room temperature for 30 to 60 min under constant or repeated stirring.

### Separation of labelled proteins

The labeled protein can be separated from unreacted dye by gel permeation chromatography, e.g. using a Sephadex™ G-25, G50 or Bio-Gel™ P-10 column. We recommend to use Sephadex™ G-25. The column should have a diameter of at least 1 cm and a length of 12 cm. It can be equilibrated with phosphate buffer of pH 7.2 (22 mM) or another buffer of choice. The same buffer can be used for elution. Usually, the first fluorescent band is the labelled protein, while free dye will elute in a second fluorescent band.

In case you have to work with diluted samples, you may purify the conjugate by extensive dialysis. But this is less efficient and not as fast as purification by gel filtration.

### Storage of conjugates

In general, conjugates should be stored under the same conditions used for the unlabeled protein. For storage in solution at 4 °C, 2 mM sodium azide can be added as a preservative. Typically, protein conjugates will be stable for several months. For long-term storage, aliquots may be frozen at -20 °C to avoid repeated freezing and thawing. Protect from light. If your protein tends to instability, please use one of our BioStab solutions specially designed for stabilization of proteins.

After long-term storage of conjugate solutions, we recommend to centrifuge in a micro-centrifuge before use. This will remove any aggregates which might have formed.

### Directions for oligo-nucleotides with Atto 465 NHS ester

- Prepare a solution of 0.1 mM solution (e.g. 5 nmol in 50 µl) of amino-modified oligo-nucleotide in carbonate buffer (0.2 M, pH 8-9).
- Prepare a solution of 5 mg/ml activated label in anhydrous DMF.
- Add ~ 50 µl of oligo-nucleotide solution to 30 µl of label solution.
- Incubate the reaction at room temperature for 2 hours under shaking. If longer reaction times are required, the pH value should be diminished to pH 7-7.5.

### Separation of labelled oligo-nucleotides

Conjugated oligo-nucleotides can be separated from free dye using gel filtration or reversed phase HPLC. Sephadex is a registered trademark of GE Healthcare

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

