

## 53394 Atto 637 NHS

### Application

Atto 637 is a suitable dye for the red spectral region. The dye is designed for application in the area of life science e.g. labeling of DNA, RNA or proteins. Atto 637 has a strong absorption, high fluorescence quantum yield, high photostability, excellent water solubility and little triplet formation. The dye has a zwitterionic characteristic.

### Product Description

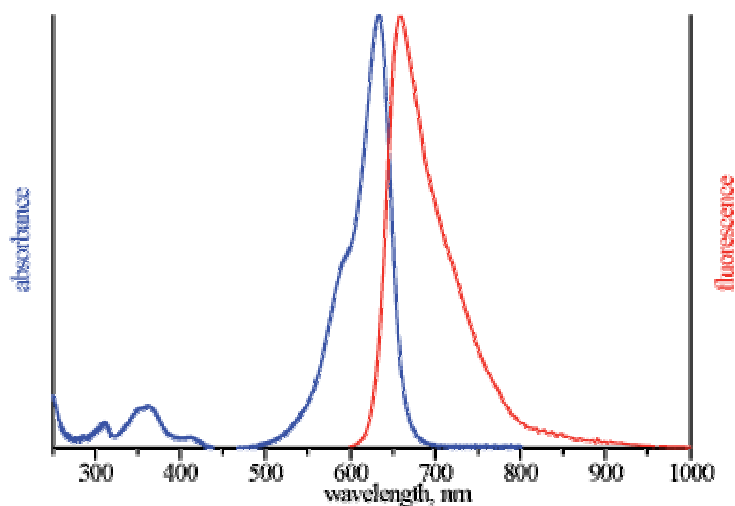
#### Atto 637

$\epsilon$	120.000 x M <sup>-1</sup> cm <sup>-1</sup>
$\eta_{fl}$	25% (in water)
Abs. max.:	635nm(H <sub>2</sub> O)
Em. max.:	659nm(H <sub>2</sub> O)
$\tau_{fl}$	1.9 ns
MW:	838g / mol
Quantity	1 mg

#### Atto 637 NHS

MW 608 g / mol

Absorption and Emission (arbitrary units)



### Directions for labelling of proteins with Atto 637 NHS ester

Dissolve protein in bicarbonate buffer (0.1 M, preferably of pH 7.5) 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 637 NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto 637 NHS in 500  $\mu$ l). 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. Therefore we recommend to try 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  $\mu$ l of dye solution to 1 ml protein solution.

Incubate the reaction at room temperature for 30 to 60 min under constant or repeated stirring.

In case you work with higher pH for labelling, adjust pH of solution to neutral pH values immediately after labelling.

### Separation of labelled proteins

The labelled 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 an other 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 637 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 7.5). Prepare a solution of 5 mg/ml activated label in anhydrous DMF. Add appr. 50 µl of oligo-nucleotide solution to 30 µl of label solution. Incubate the reaction at room temperature for at least 4 hours under shaking.

### Separation of labeled oligo-nucleotides

Conjugated oligo-nucleotides can be separated from free dye using gel filtration or reversed phase HPLC.