Fluka

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12366 Fluorescent red 633 reactive

Fluorescent red 633 reactive is an aminoreactive fluorescent biolabel designed for excitation by red lasers (He/Ne; 633 nm) and diode lasers (635 – 650 nm). It can be used for covalent coupling to proteins and other biomolecules containing primary amino groups like amino modified DNA-oligomers and amino modified biotin. Fluorescent red 633 reactive exhibits a strong solid state emission. With an negative over all charge Fluorescent red 633 offers improved water solubility.

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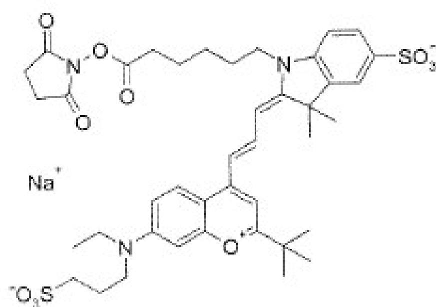
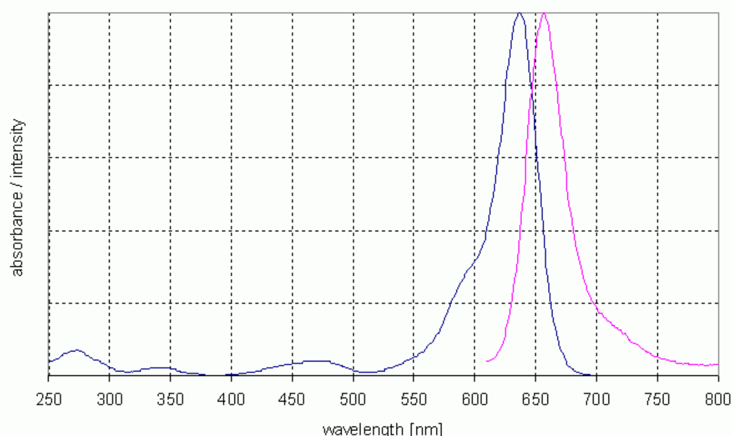
Product Description

Net formula	$C_{41}H_{50}N_3O_{11}S_2Na$
MW	847.98 $g{\cdot}mol^{-1}$
Appearance	deep blue solid
Solubility	soluble in water, methanol, DMF, DMSO
Molar absorbance	200.000 $l{\cdot}mol^{-1}{\cdot}cm^{-1}$ (determined in DMF)
Quantity	1 mg



Spectrum



Directions for labelling of proteins with Fluorescent red 633 ester

Dissolve protein in of 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 7.5, for each ml of dialyzed antibody solution.

Dissolve Fluorescent red 633 reactive in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Fluorescent red 633 reactive in 500 µl). This solution should always be prepared immediately before conjugation.

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As number and position of amine groups vary between different proteins, the optimum of dye/protein ratio also varies. Wherefore 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 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.
In case you label with pH values higher as recommended, 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 unstability 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 Fluorescent red 633 reactive
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.