

Product Information

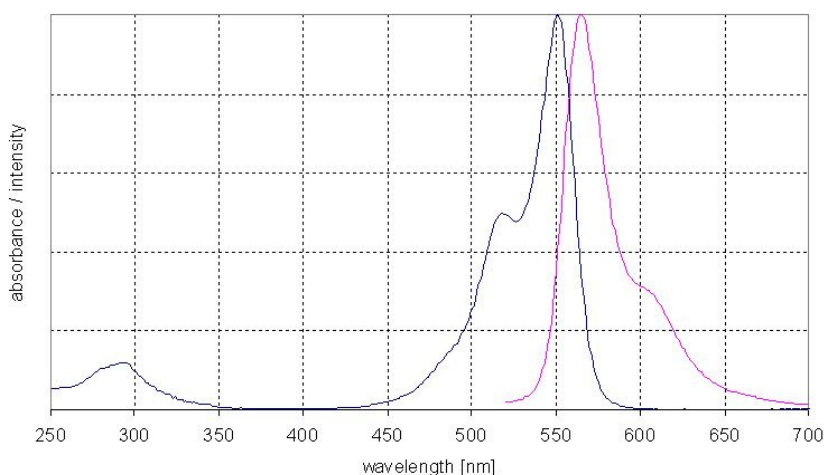
72712 Fluorescent orange 547 reactive

Fluorescent orange 547 reactive is an aminoreactive fluorescent biolabel designed for excitation by He/Ne lasers (at 544 nm) or mercury arc lamps. 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 orange 547 reactive exhibits a strong solid state emission. With an negative over all charge Fluorescent orange 547 offers improved water solubility.

Product Description

Net formula	$C_{34}H_{38}N_3NaO_{10}S_2$
MW	$735.80 \text{ g}\cdot\text{mol}^{-1}$
Appearance	violet powder
Solubility	soluble in water, methanol, DMF, DMSO
Molar absorbance	$150.000 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ (determined in DMF)

Spectrum



Directions for labelling of proteins with Fluorescent orange 547 reactive ester

Dissolve protein in 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 orange 547 reactive in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Fluorescent orange 547 reactive in 500 μ 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. 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 μ 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 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 SephadexTM G-25, G50 or Bio-GelTM P-10 column. We recommend to use SephadexTM 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 Fluorescent orange 547 reactive

Prepare a solution of 0.1 mM solution (e.g. 5 nmol in 50 μ l) 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 μ l of oligo-nucleotide solution to 30 μ l 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.

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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.