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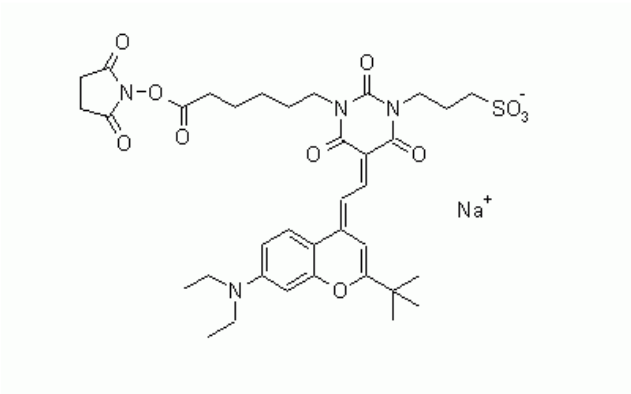
Product Information

92592 Fluorescent orange 550 reactive

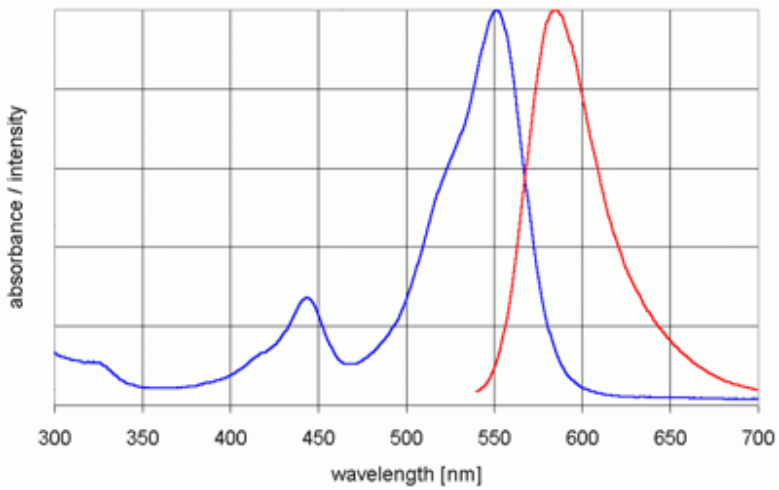
Fluorescenct orange 550 reactive is a new fluorescent dye expecially well suited for excitation by He-Ne-lasers (at 544 nm) or mercury arc lamps. Fluorescent orange 550 reactive shows strong fluorescence and may be used as alternative cyanine dyes.

Product Description

Net formula	$C_{36}H_{47}N_4NaO_{11}S$
MW	766.83
Appearance	deep red solid
Solubility	soluble in DMF
Molar absorbance	120.000 l· mol-1· cm-1 (determined in ethanol)
Abs. Max	553 nm (Ethanol) ; 550 nm (0.1 M phosphate, pH 7.0)
Emission Max.	578 nm (Ethanol) ; 590 nm (0.1 M phosphate, pH 7.0)



Spectrum



Directions for labelling of proteins with Fluorescent Orange 550 reactive

1. To prepare a stock solution of the label, dissolve 1 mg of label (NHS-ester) in 50 µl absolute, amine-free DMF (final concentration: approx. 25 nmol· μl^{-1}).
2. Dissolve the desired amount of protein in bicarbonate buffer (pH 9.0, 50 mM), e.g. 1 mg of avidin in 200 µl buffer. Protein concentrations should typically be 2 mg/ml or higher. For antibodies, dialysis (e.g. two changes of buffer, one hour dialysis for each step) is recommended.
3. Transfer an appropriate volume of the label stock solution to the protein solution dropwise and under stirring. Due to the high reactivity of the NHS ester add an equimolar amount or up to an double excess of label to the protein to obtain a dye to protein ratio (D/P) between 1 and 2. Higher molar excesses of the label can lead to overlabelling of the protein causing a decrease in quantum yield of the conjugate.
4. Incubate the mixture, react for one hour at room temperature.
5. Separate the obtained protein conjugate from unreacted free dye using a Sephadex column (Sephadex G25 medium; eluent PBS pH 7.2, 22 mM. Cat. no. 76847). For 1 mg of labelled protein, a column of at least 20 cm length and e.g. 6 mm width is a very good choice. First coloured band is the labeled protein.

Bicarbonate buffer, pH 9.0, 50 mM
Dissolve 2.1 g of NaHCO3 in 400 ml double distilled water. Adjust the pH to 9.0 by carefully adding small volumes of 1 M HCl or 1 M NaOH while controlling pH with a pH-meter. Add double distilled water up to a final volume of 500 ml.

Storage:
store at -20°C

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.