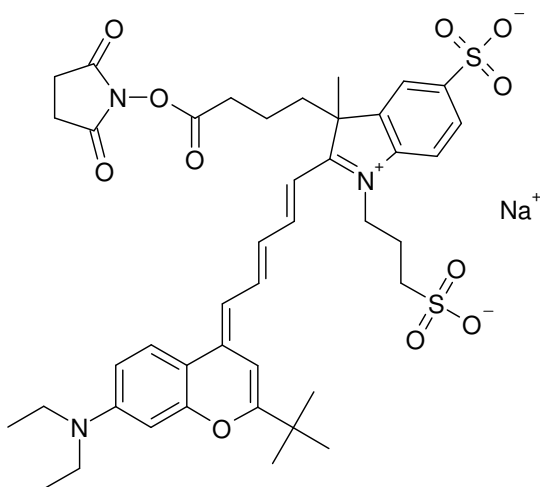


56794 Fluorescent Red 731 Reactive

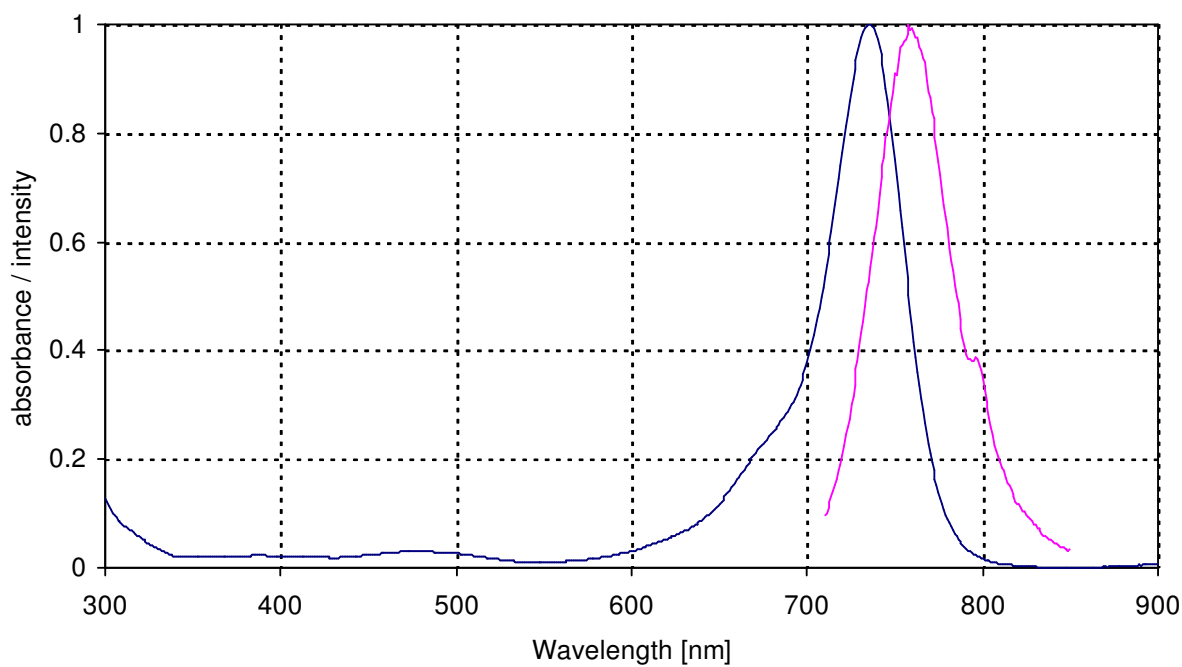
Fluorescent red 731 reactive is a new fluorescent label for the near infrared area. Extremely long wavelength fluorescence is especially well suited for applications where sample autofluorescence might be critical or penetration of tissue or other matrix is required. Fluorescent red 731 reactive shows strong fluorescence.

Product Description

MW	859.99
Molar absorbance	225.000 l · mol ⁻¹ · cm ⁻¹ (determined in ethanol)
Abs. Max	736 nm (Ethanol)
Emission Max.	759 nm (Ethanol)
Quantity	1 mg



Spectrum



Directions for labelling of proteins with Fluorescent Red 731 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. $25 \text{ nmol} \cdot \mu\text{l}^{-1}$).
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 a 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. Fluka 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 NaHCO_3 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.