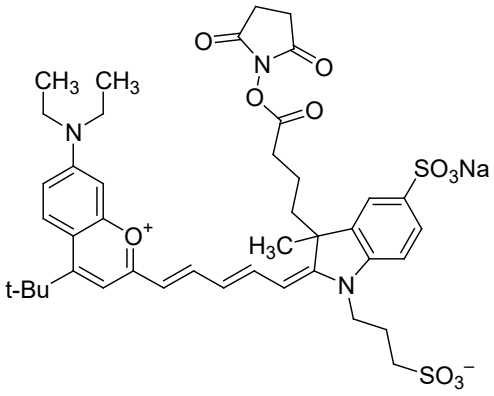
40436 Fluorescent Red NIR 781 Reactive

Application

Fluorescent red 781 reactive is a new fluorescent label especially well suited 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. Fluorescence NIR 781 reactive shows strong fluorescence. This holds also for its solid state emission.

Product Description

Net Formula	$C_{42}H_{50}N_3O_{11}S_2Na$
MW	860.00
Solubility	Methanol, ethanol, DMF, DMSO
Molar absorbance	170.000 l· mol-1· cm-1 (determined in ethanol)
Abs. Max	783 nm (Ethanol)
Emission Max.	800 nm (Ethanol)
Quantity	1 mg



Directions for labeling of proteins with Fluorescent Red 781 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.
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 overlabeling of the protein causing a decrease in quantum yield of the conjugate. See table for the appropriate volume in dependence of the molecular weight of selected proteins.
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. Product No. 76847). The 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.

Protein	**Protein A**	**Strept-avidin**	**Avidin**	**IgG**	**IgA**	**IgE**
MW [g·mol-1]	42000	60000	67000	150000	160000	190000
D/P = 1 [µl]	0.75	0.52	0.48	0.22	0.20	0.16
D/P = 2 [µl]	1.50	1.06	0.95	0.42	0.40	0.35
D/P = 3 [µl]	2.18	1.58	1.42	0.64	0.59	0.53
D/P = 4 [µl]	3.01	2.12	1.89	0.84	0.79	0.66
D/P = 5 [µl]	3.76	2.65	2.36	1.06	1.00	0.82

Table: Suggestions for aliqouts of a 50 µl stock solution of label solution in DMF (1 mg) to be added to 1 mg of protein dissolved in bicarbonate buffer (50mM, pH 9.0) in dependence of the desired D/P ratio

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.