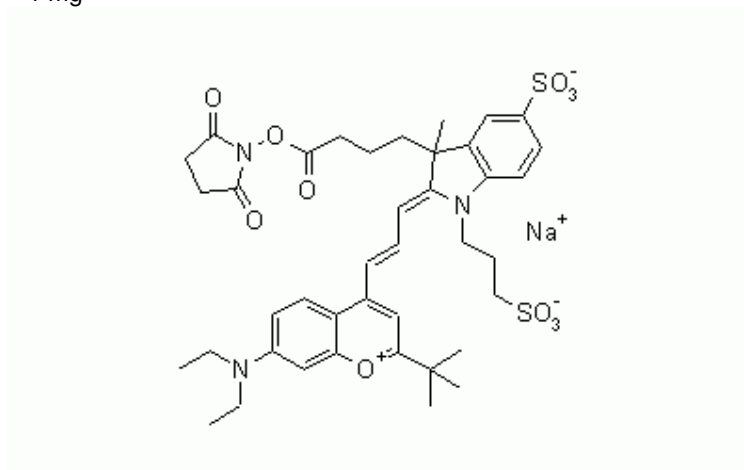


## 12326 Fluorescent Red 631 Reactive

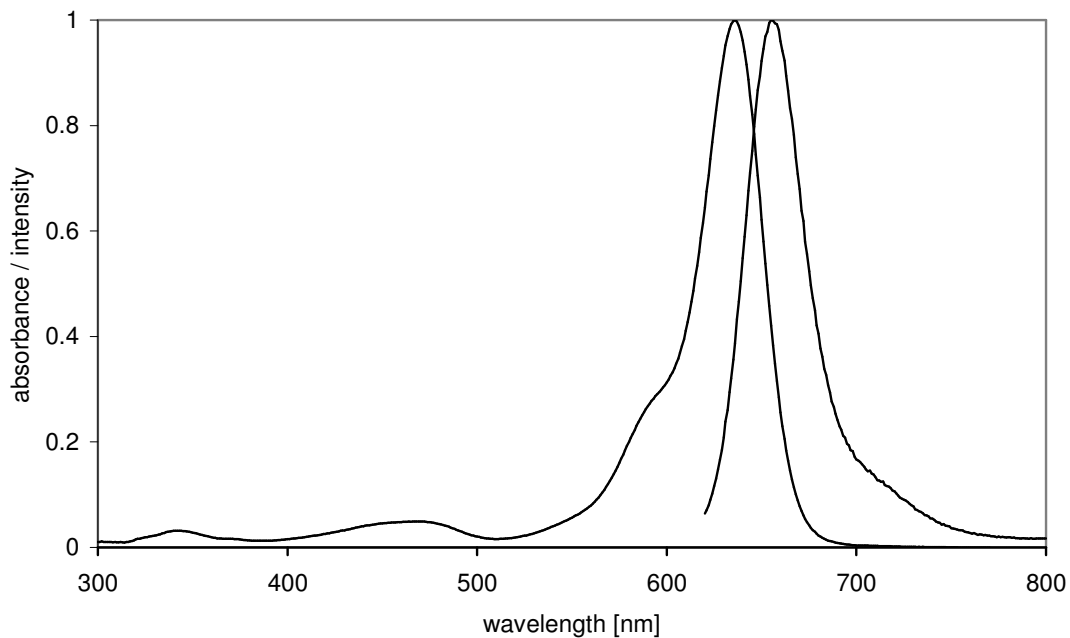
Fluorescent red 631 reactive is a new fluorescent label especially well suited for excitation by He-Ne-lasers (at 633 nm). Fluorescent red 631 reactive shows strong fluorescence.

### Product Description

Net formula	$C_{40}H_{48}N_3O_{11}S_2Na$
MW	833,95
Molar absorbance	185.000 l · mol <sup>-1</sup> · cm <sup>-1</sup> (determined in ethanol)
Abs. Max	637 nm (Ethanol)
Emission Max.	658 nm (Ethanol)
Quantity	1 mg



### Spectrum



**Directions for labeling of proteins with Fluorescent Red 631 reactive**

1. To prepare a stock solution of the label, dissolve 1 mg of label (NHS-ester) in 50  $\mu$ l absolute, amine-free DMF (final concentration: approx. 25 nmol  $\cdot$   $\mu$ l<sup>-1</sup>).
2. Dissolve the desired amount of protein in bicarbonate buffer (pH 9.0, 50 mM), e.g. 1 mg of avidin in 200  $\mu$ l 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 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 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. Fluka no. 76847). The first coloured band is the DY-labeled protein.

**Bicarbonate buffer, pH 9.0, 50 mM**

Dissolve 2.1 g of NaHCO<sub>3</sub> 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 <sup>-1</sup> ]	42000	60000	67000	150000	160000	190000
D/P = 1 [ $\mu$ l]	0.99	0.695	0.62	0.28	0.26	0.22
D/P = 2 [ $\mu$ l]	1.98	1.39	1.24	0.56	0.52	0.44
D/P = 3 [ $\mu$ l]	2.97	2.085	1.87	0.84	0.78	0.66
D/P = 4 [ $\mu$ l]	3.96	2.78	2.49	1.11	1.04	0.88
D/P = 5 [ $\mu$ l]	4.95	3.48	3.11	1.39	1.3	1.1

Table: Suggestions for aliquots of a 50  $\mu$ l 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