# 55536 Fluorescence red Mega 480 NHS ester

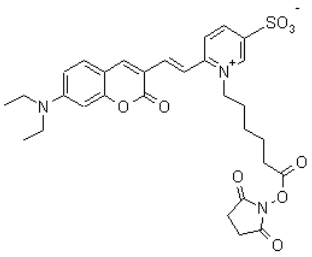
## Application

Fluorescent red Mega 480 NHS ester and other Mega labels are specifically designed for multicolour techniques. It is characterized by an extremely large stoke's shift between excitation and emission maxima. This label is especially well suited for excitation by argon lasers or other short wavelength excitation light. This also means, that it can be excited with the same light source or filter arrangement than fluorescein and other widely used dyes as well as other Mega labels. Due to the different emission wavelength several different labels can be detected independently in the same experiment. Due to the small size of the Mega-labels and the direct conversion of excitation light into emission without using a FRET mechanism we foresee a variety of possible applications including DNA sequencing and FISH microscopy.

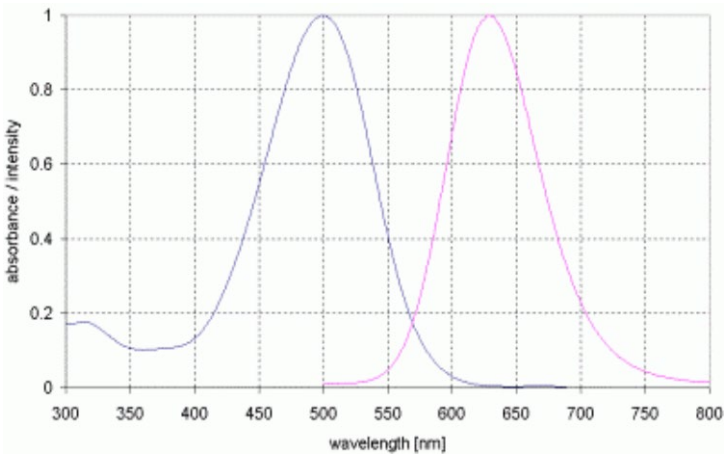
## Product Description

| | |
|---|---|
| Formula | $C_{30}H_{33}N_3O_9S$ |
| MW | 611.66 g/Mol |
| ε | 40.000 |
| Abs. max.: | 500nm($H_2O$) |
| Em. max.: | 630nm($H_2O$) |

## Structure



## Fluorescence spectra



**Directions for labeling of proteins with Fluorescent Red Mega 480 NHS**

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. 40 nmol· $\mu l^{-1}$).
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.
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. Cat. no. 76847). The first coloured band is the DY-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.

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.