**Product Data Sheet for Mfluo NIR 865**

**Catalog Number: MF-865-1MG (1 vial, 1 mg)**

## Description

MediLumine's Mfluo NIR 865 amine reactive dye has an excitation spectra peak of 863 +/- 4 nm and emission peak at 896-1 +/- 4 nm. The dye is ideal for near infrared imaging applications such as NIR I and NIR II in vivo imaging. The dye can also be conjugated to proteins by reacting an NSH ester with the primary amines on a protein

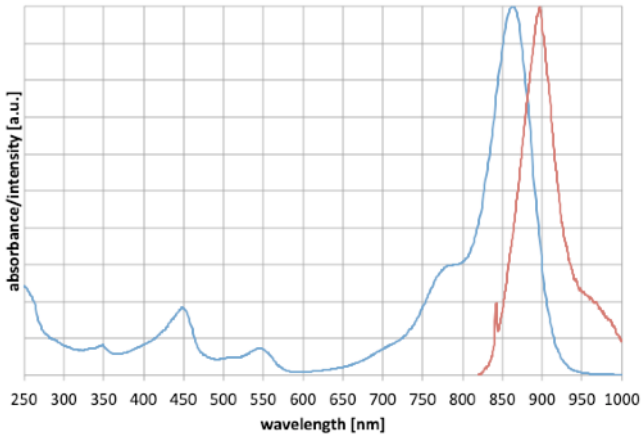
## Characteristics

**Absorption/Emission Max.:** 863 / 896 nm
**Amount:** 1 mg
**Molar Absorbance:** 170,000 M-1cm-1
**Solubility:** water, methanol, DMF, DMSO

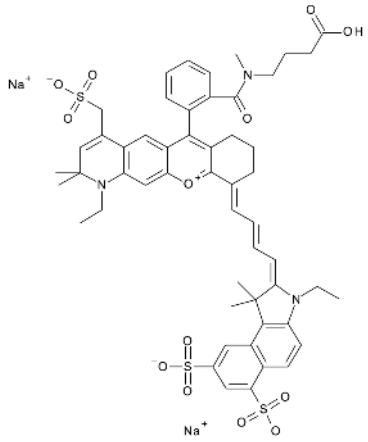
## Storage

This product should be stored at 4°C in dark. The dye is also moisture sensitive and so it should be stored in dry.

## Emission (Blue) and Excitation (Red) Spectra




## Chemical Structure



## Recommended Dosage for In Vivo Imaging

The recommended dosage for in vivo imaging is 1 mg per 1000 grams. The recommended maximum injection volumes are 200 µl for intravenous injections, 2 ml for intraperitoneal injections and 1 ml for sub-cutaneous injections.

## Recommended Materials for Protein Conjugation

– Protective gloves
– 1 pipet 0.5–10 µl and corresponding tips (white)
– 1 pipet 10–100 µl and corresponding tips (yellow)
– Centrifuge for Eppendorf vials
– Ultrasonification bath (recommended, but not required)
– 1 Pasteur pipet
– Dry DMF (e.g. Fluka PO-no. 40228)
– Sephadex G-25 medium (GE Healthcare)
– Column for size exclusion chromatography (e.g. 5 ml or 10 ml graduated pipettes)
– Silanized glass fiber wadding (e.g. Macherey & Nagel PO-no. 718 002)
– 1 glass bar (at least as long as the column)

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– 4 beakers (size can vary)
– 1 tripod with clamp
– Shaker for Eppendorf tubes
– 2 Eppendorf vials (1.5 ml)
– UV-spectrometer

*Only when dialysis is required:*
– QuixSep Micro dialyzer (Orange Scientific n.v./s.a./ Belgium)
– Dialyzing membrane (also avaibable from Orange Scientific)
– Magnetic stirrer
– Stirring bar

**Reagent Preparation for Protein Conjugation**

Sodium bicarbonate buffer for dialysis and labelling (10x /500 mM). Dissolve 21 g (250 mmol) of sodium hydrogen carbonate in 400 ml distilled water. Add 1 g of sodium azide (0.2 per cent, works as bacteriozide). Adjust the pH with a concentrated aqueous solution of sodium hydroxide to pH 8.3. Prior to use, dilute the buffer by adding 9 parts water to one part of the concentrated stock solution (v/v).

100 mM PBS-buffer pH 7.4 for elution and storage. Dissolve 2.19 g sodium dihydrogen phosphate (NaH2PO4·H2O, 15.9 mmol), 14.97 g disodium hydrogen phosphate (Na2 HPO4·2H2O, 84.1 mmol), 5.8 g sodium chloride (100 mmol) and 0.32 g sodium azide in 1 l distilled water.

**Method for Protein Conjugation**

To start the labelling reaction, transfer 3 µl of the NHS-ester solution to the IgG in the reaction buffer. If you can control the concentration of the protein, a concentration of 5 mg/ml is recommended. Lower concentrations lead to a reduced labelling efficiency due to the fact that the hydrolysis of the NHS-ester competes the labelling reaction, and the less protein is present the more the hydrolysis dominates.

Allow the labelling to be done in the Eppendorf vial placed in a shaker over the course of two hours. After this time, the NHS-ester should be either bound to the protein or have been hydrolized which makes the use of a stopping reagent obsolete. The Sephadex G-25 medium is a classic gel filtration material. Upon addition of elution buffer, it swells by a factor of five in volume. Thus, it is recommended to put at least 6 ml of elution buffer onto 1.2 g Sephadex in a beaker for filling a 5 ml-column. Double the amounts for a 10 ml column. A 5 ml-column is recommended for reaction solution volumes up to 250 µl. Please use a 10 ml-column for volumes up to 1 ml.

To prepare the size-exclusion chromatography column, fix the column vertically in the clamp of the tripod. Put a small amount of the silanized glass fiber wadding into the column and push it with the help of the glass bar to the small opening at the ground. The wadding allows the buffer to flow through it while it holds back the gel. Pipet once elution buffer into the column, immediately followed by the swollen gel in elution buffer which had been allowed before to equilibrate for at least one hour. Fill the column entirely with the gel and allow thereby the material to settle. If the Sephadex in the beaker runs dry due to the aspiration of the buffer, simply add some more of the elution buffer to liquidize the gel again. Keep a 1 cm region at the top of the column free of the gel.

Now transfer your coloured labelling solution slowly onto the column and allow it to sink into the gel. Wash your pipet carefully (or use a new one) to remove all traces of dye or protein which would contaminate your elution buffer.

After the labelling solution has sunk fully into the column, start to elute the conjugate by slowly adding the elution buffer drop by drop onto the column. You can add bigger portions once there is a dye free zone on top of the column. When the reaction solution is eluated, you will see a separation between the labelled protein which runs ahead as a quite sharp band while the free dye is slowly smearing behind. Once the conjugate arrives at the bottom of the column collect it in an Eppendorf vial.

While selecting the size of the vial keep in mind that the volume of the protein conjugate solution increases by a factor of ca. 2.5 on the column. Dispose the used Sephadex gel after collecting the conjugate and wash the column. It would be very time-consuming to wash the free dye from the gel, and traces of it could impurify the next conjugate. It is really not worth the time, and the price for 1 g Sephadex is less than $5 USD. To characterize your protein conjugate solution it is recommended to record an absorption spectrum of it over the full wavelength range covering both the protein absorption at 280 nm as well as the maximum wavelength of the dye together with the shape of the dye's absorption band. Avoid thereby absorbance values exceeding "2" because such values are hard to interpret. Use cuvettes with smaller path-lengths instead or dilute a small fraction of your conjugate solution appropriately with the elution buffer, e.g. by a factor of 20.

**Product Safety and Handling**

This product is for R&D use only, not for drug, household, or other uses. Please review the material safety datasheet (MSDS) for proper safety and handling procedures

**Ordering Information**

For ordering call 1 844 360 1574 or visit us online at www.medilumine.com

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