



Fluidic
Analytics

fluidiphore **rapid** amine 503

Labelling kit user manual

Catalog Number L00487

Protocol for labelling proteins:

1. Dissolve the dry fluidiphore dye in 100 μL of dimethylformamide (DMF), methanol or acetonitrile. Do not use amine-containing solutions or buffers as a solvent.
2. Dissolve 2 mg of your protein in 0.5 mL of bicarbonate buffer (0.1 M, pH 8.3). Alternatively transfer the protein into bicarbonate buffer (0.1 M, pH 8.3) by buffer exchange.
3. Add 5 – 10 μL of the dye solution drop-wise.
4. Mix well by pipetting up and down and allow the mixture to stand for 30 minutes at room temperature, until blue color has disappeared. The solution will turn red or yellow, dependent on the pH (see the next page for examples of the colour change).
5. Centrifuge your sample at 14 000 $\times g$ for 15 minutes to remove dye precipitate.

Purification:

For some applications the purification of the dye conjugated protein may be necessary.

To purify use size-exclusion chromatography with Sephadex G25 as stationary phase and a buffer of choice as the eluent. The red band indicates the labelled protein.

Bicarbonate buffer:

Dissolve 4.2 g of NaHCO_3 in 500 mL doubly distilled water. Adjust the buffer to pH 8.3 with 1 M NaOH (the dye is much more reactive in a pH range of 8.0 - 9.0).



Technical specifications:

Chemical properties: 1 mg lyophilized blue solid which is soluble in DMF, methanol and acetonitrile. DMSO can also be used but is not recommended.

Absorption: 503 nm (conjugated), 612 nm (free)

Emission: 600 nm (conjugated), 665 nm (free)

ϵ L/(mol cm): 24,000 (conjugated), 60,000 (free)

Quantum Yield: ~50% (conjugated), less than 1% (free)

Storage: The lyophilized dye should be stored at 4 °C. The reconstituted dye should be stored at -20 °C sealed in a bag with desiccant. Allow to reach room temperature before taking out of the bag. Unreacted dye can be stored for up to 6 months.

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