Data Sheet

Jena Bioscience

Mant Protein Labeling Kit

Fluorescent labeling of primary amino groups

Cat.-No.	Amount
FP-201-MNT	10 reactions

For *in vitro* use only!
Protect from light – work under low light conditions!

Kit Contents

- **Mant NHS-ester**
  1 vial containing 1 mg
- **Dimethylformamide (DMF)**
  500 µl
- **Sodium bicarbonate**
  1 vial containing 84 mg
- **Ultra-pure water**
  1 ml

Storage and Stability

Upon receipt store the dye at –20°C. The other components may be stored at room temperature.
If stored as recommended, Jena Bioscience guarantees optimal performance of this product for 12 months.

Spectroscopic data of Mant

Excitation maximum: λ_{Ex} = 335 nm
Emission maximum: λ_{Em} = 440 nm
Extinction coefficient: ε_{max} = 2,700 cm^{-1} M^{-1}

Application

Fluorescence techniques have become a major tool in biological sciences. Fluorescent proteins such as GFP and DsRed fused to the protein of interest (POI) allow expression analysis and *in vivo* protein localization. Their application however, is limited due to the large molecular weight of those fusion proteins. Furthermore, the associated molecular biology is tedious and time-consuming.
Small fluorophores, covalently attached to the POI, may help to overcome this problem.
The mant fluorophore is sensitive to its environment and therefore a convenient tool to study protein-protein interactions as well as protein dynamics.
Upon binding to a protein, the fluorescence quantum yield of the mant fluorophore increases, thus leading to an increase of fluorescence.[1,2] Changes in the solvent polarity may also be observed by altered fluorescence properties of mant.
Another application of mant labeled proteins is FRET (fluorescence resonance energy transfer). The energy of excited aromatic amino acids, most notably tryptophan and tyrosine residues, is transferred to proximate mant fluorophores, thus allowing investigation of binding parameters of various receptors, enzymes and protein complexes.
Mant is perfectly suited for many biological applications due to its low molecular weight and minimal steric hindrance.
This Jena Bioscience Protein Labeling Kit is designed for labeling the lysines of a POI with a mant fluorophore resulting in a fluorescent protein-fluorophore conjugate. It contains all reagents required for performing 10 separate labeling reactions of 1 mg of POI.

[1] Rojas *et al.* (2003) Established and Emerging Fluorescence-Based Assays for G-Protein Function: Ras-Superfamily GTPases. *Combinatorial Chemistry & High Throughput Screening* **6**: 77-99

[2] Hanzal-Bayer et al. (2005) Properties of the Interaction of Arf-like Protein 2 with PDEδ. J. Mol. Biol. **350**: 1074-1082

Protocol

General notes

The protein concentration should be at least 2 mg/ml since labeling efficiency suffers from lower concentrations. We recommend using about 1 mg protein per labeling reaction.

Buffers containing primary amines such as Tris and glycine are not suitable for the labeling reaction and must be exchanged with suitable amine-free buffer such as PBS, MES, or HEPES before starting the labeling reation.

Experimental protocol

1. Dissolve the sodium bicarbonate by adding 1 ml ultra-pure water. The resulting 1 M solution is stable at 4°C for at least 2 weeks.
2. Add the appropriate volume of sodium bicarbonate (1 M) to your protein solution to achieve a final concentration of 100 mM.
3. Prepare the dye by adding 500 µl DMF resulting in a dye concentration of 2 mg/ml. Vortex until the NHS ester is completely dissolved! We recommend preparing the solution shortly prior to use however, it is stable for two months at –20°C. Whenever handling the fluorophore or conjugate, work under low light conditions!
4. Add 500 µl protein solution (2 mg/ml) and 50 µl dye (2 mg/ml) to an appropriate vial. Vortex carefully and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
5. Incubate for one hour in a shaker at room temperature. Protect from light!
6. Purify the conjugate using standard gel filtration columns such as Sephadex G-25 or similar. Alternatively, the free dye may be separated from the conjugate by dialysis or appropriate spin concentrators.

   Please note that protein purification materials are not provided with the kit!

Troubleshooting

Inefficient Labeling

- Concentration of protein solution
  The assay is optimized for labeling of 1 mg protein at a concentration of 2 mg/ml.
  Increase the amount of dye proportionally for protein concentrations above 2 mg/ml. If your protein concentration is very low (< 1 mg/ml) apply spin concentrators to achieve a final concentration of 2 mg/ml.
  The efficiency of labeling is strongly concentration dependent and varies among different proteins. Thus, in every single case optimization might be necessary to obtain the desired degree of labeling.
- Buffer composition
  Protein solutions containing primary amines (even traces thereof) dramatically decrease labeling efficiency. Make sure that your protein is extensively dialyzed in case it has been in contact with amine-containing substances.
- Impact of the pH
  Check the pH of your protein solution! The reference range is 8.2 – 8.5.
  The primary amino groups of the protein must not be protonated to be reactive thus, the pH of the protein solution has to be sufficiently high. On the other hand, the hydrolysis rate of NHS esters increases with the pH of the solution, resulting in non-reactive dye. Optimal labeling results have been obtained at pH 8.3.
  100 mM sodium bicarbonate might not be sufficient to raise the pH to optimum in strongly buffered protein solutions at a lower pH. You may add more sodium bicarbonate until the optimal pH is achieved.

Note that labeling efficiency not only depends on the surrounding conditions but also on the protein characteristics. The tertiary structure and the resulting number of lysines on the surface of the protein play a role as well as the isoelectrical point and the behaviour of the protein at pH 8.3.

Overlabeling

Reasons for overlabeling can be a high number of lysines on the surface of your protein and/or optimal characteristics of the protein at pH 8.3.
To prevent overlabeling, decrease the amount of dye or increase the protein concentration. You may also reduce the reaction time.

Purification of the conjugate

The dye is unstable in aqueous solutions – the hydrolysis rate of NHS esters increases with the pH of the solution. Thus, a certain amount of free dye is

produced in each labeling reaction and needs to be separated from the conjugate.
If your purified conjugate still contains traces of free dye apply it to a second purification step. Check the purity of your conjugate by SDS-PAGE or thin layer chromatography.
Separation of free dye from the conjugate becomes more difficult at higher concentrations of free dye. The column or membrane might even be blocked by the dye at very high concentrations, so try to optimize the labeling reaction in order to decrease the concentration of free dye. Keep in mind that overall yield suffers from additional purification steps.

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