Cat Nr: L004

Monolith NT™ Protein Labeling Kit RED - MALEIMIDE

MicroScale Thermophoresis Grade

Kit for 1 h labeling of proteins with the RED fluorescent dye NT-647-MALEIMIDE for use in MicroScale Thermophoresis

CONTENT AND STORAGE

Monolith NT™ Protein Labeling Kits are shipped at room temperature. Each kit contains material sufficient for 4 protein labeling reactions. Depending on the amount of protein used, enough material for approximately 1000 MicroScale Thermophoresis experiments can be prepared.

4* 10 µg NT-647- MALEIMIDE dye **[store at -20 °C]**
4* Spin Column A, Buffer Exchange **[store at 4 °C]**
4* Gravity Flow Column B, Purification **[store at 4 °C]**
2* Adapter for 15 ml Falcons
4* Labeling Buffer **[store at 4 °C]**

Expiry date: see kit cover

ADDITIONAL MATERIAL REQUIRED

- Variable speed benchtop microcentrifuge
- 1.5 – 2 ml microcentrifuge collection tubes
- Assay buffer (buffer of choice, e.g. PBS)
- 100 % DMSO
- Aq. dest.

PROTEIN LABELING PROCEDURE

The Monolith NT™ Protein Labeling Kit provides convenient means for labeling small amounts (2 – 20 µM) of purified protein with our NT-647-MALEIMIDE fluorescent dye. This kit has been optimized for labeling proteins with molecular weights higher than 5 kDa, and contains everything needed to perform four labeling reactions and to separate the resulting conjugates from excess dye. Convenient gravity flow and spin columns are used to purify the labeled protein with high recovery yields (60 - 90 %) depending primarily on the molecular weight of the starting material. Labeling and purification can be completed in less than 60 minutes.

The NT-647-MALEIMIDE reactive dye contains Maleimide chemistry, which reacts efficiently with sulfhydryl groups of proteins to form highly stable dye-protein-conjugates. Sulfhydryl groups are found on cysteine residues which are usually solvent accessible and therefore suitable for labeling reactions. NT-647-MALEIMIDE dye labeled proteins show fluorescence excitation and emission maxima of approximately 650 nm and 670 nm, respectively.

IMPORTANT INFORMATION BEFORE STARTING

The labeling chemistry requires that the protein is dissolved in a suitable labeling buffer at the correct pH.

The purified protein should be in a buffer that **does not** contain sulfhydryl groups (glutathione). The reducing agents DTT, DTE and β-mercaptoethanol should also be avoided (see paragraph below for information on reducing agents). All of these substances will significantly reduce protein labeling. Also, partially purified protein samples or protein samples containing carriers like BSA will not be labeled properly and should not be used.

If all cysteines are in the form of cystine disulfides then reduction using DTT or β-mercaptoethanol prior to reaction is necessary to generate the reactive cysteine. The reducing agent must then be removed through dialysis / gel filtration prior to reaction with the dye. Alternatively, TCEP can be used to reduce the cystine disulfides and does not need to be removed. However, DTT and β-mercaptoethanol are better suited than TCEP for the subsequent MST experiment, since TCEP may in some cases reduce reproducibility.

Step A (buffer exchange) can be avoided if the protein sample is purified directly into a suitable buffer (good buffers are HEPES, PBS, Na-Ac) with no imidazole, DTT or β-mercaptoethanol.

Please note: If another labeling buffer is used other than that supplied, a higher excess of dye might be required for good labeling efficiency.

If the protein sample is not in a suitable buffer then **proceed with Step A (buffer exchange)**.

If the protein sample is in a buffer compatible with the labeling reaction then **skip Step A and go straight to Step B (labeling)**.

Step A
Buffer Exchange

1. Add 3.0 ml aq. dest. to the vials containing the Labeling Buffer salt.
2. Invert Spin Column A to suspend slurry.
3. Twist off bottom (twist slightly in both directions).
4. Remove the cap of the column.
5. Place column in 1.5 – 2 ml microcentrifuge collection tube.
6. Centrifuge at **1500 x g** for **1 minute** to remove excess liquid.
7. Add 300 µl of labeling buffer and repeat steps 5 and 6, three times.
8. Place 40-100 µl protein in the **center** of the resin. Be careful not to disturb the resin or allow the sample to flow around the resin bed.
9. Place the sample in new microcentrifuge collection tube and centrifuge at **1500 x g** for **2 minutes.**

Step B
Labeling

For protein labeling

1. Adjust the protein concentration to 2 - 20 µM using the Labeling Buffer and use 100 µl of protein solution. For calculation of the molarity of your sample use the following equation:

   c(Protein) [Mol/l] = c(Protein) [mg/ml] / MW (Protein) [Da]

2. Add 30 µl of 100 % DMSO to the solid fluorescent dye (i.e. yielding approx. **470 µM** solution).[1]
3. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
4. Prepare 100 µl of dye solution (in **Labeling Buffer**) with a concentration of 2 - 3 x than your protein concentration.[2]
5. Mix protein and dye in a 1:1 volume ratio **(200 µl final volume).**
6. Incubate for 30 minutes at room temperature in the dark.
7. In the meantime, prepare Step C.

[1]We do not recommend using the dye for more than a few hours after suspending it.
[2] For some samples labeling efficiency may be increased by using a higher fold excess of dye.

STEP C
PURIFICATION

For optimal results in MicroScale Thermophoresis, unreacted “free” dye needs to be eliminated. This kit is optimized for this.

The labeling efficiency can be assessed by measuring the ratio of protein to dye spectroscopically (e.g. by measuring absorption at 280 nm for protein and 650 nm for the dye, molar absorbance: 250,000 $M^{-1}cm^{-1}$) after the clean-up procedure below. Please use a correction factor of 0.027 for the protein adsorption at 280 nm.

1. Remove the top cap of Column B and pour off the column storage solution.
2. Remove the bottom cap and place in a 15 ml tube (adapter supplied).
3. Equilibrate and wash Column B by adding 3 x 3 ml of your buffer of choice (flow through by gravity flow).
4. Add maximum 500 µl of labeling reaction to the center of Column B. Let sample enter the bed completely (when using less than 500 µl, adjust the volume to 500 µl after the sample has entered the bed by using your buffer) and discard the flow through.
5. Place in a new 15 ml collection tube
6. Add 600 µl of your buffer and collect the eluate in 100 - 150 µl fractions. The first few µl will not contain protein and can be discarded.
7. Test the elution fractions for their fluorescence intensity in the Monolith device. According to the gel filtration principle, larger particles will elute prior to smaller particles. Use the early fractions that contain sufficient amounts of labeled protein. Depending on your buffer composition, later fractions might contain free dye.

   Note: At 20 % LED/excitation power, 10 nM labeled protein should yield fluorescence intensities of approx. 100 - 200 counts in the Nano-detectors, 1 nM labeled protein should yield approx. 8000 counts in the Pico-detectors of Monolith NT.115 and Monolith NT.Automated instruments.

8. Aliquot/store your protein.

   Tip: Most labeled proteins can be stored for several weeks to months at -80 °C if split into 10 µl aliquots into PCR tubes and flash-frozen in liquid nitrogen.

Factors Affecting the Performance of the Labeling Procedure

Coupling Efficiency

1. Make sure the protein concentration is sufficiently high and in the range of 2 - 20 µM. Lower concentrations may result in loss of coupling efficiency.
2. If the sample contains >100 mM imidazole, dilution of proteins with labeling buffer prior to labeling is recommended.
3. Avoid the use of buffers with the reducing agents DTT and β-mercaptoethanol (if a reducing agent is required, use TCEP). If the protein is in unsuitable buffer, buffer exchange is required.

Purity of the Sample

1. To prevent contamination of your sample with uncoupled dye, we recommend loading the sample directly in the center of the resin bed (Column A and Column B). Do not blow out the tips and avoid the contact of the tip with the walls of the column.
2. Do not exceed recommended centrifuge time or speeds.

Yield of the Sample

1. In case of a low sample yield increase amount of sample (max volume 100 µl) or try a different buffer for purification of the protein.

SAFETY INFORMATION

DYE

Hazard statements

H318	Causes serious eye damage.

Precautionary statements

P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/ doctor.

COLUMN B

Hazard statements

H317	May cause an allergic skin reaction.

Precautionary statements

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of waste according to applicable legislation.

For more information, please consult the respective Safety Data Sheets (SDS). SDS are available from NanoTemper Technologies upon request.

Contact

TECHNICAL SUPPORT

Please get in touch with us for specific questions concerning the product performance.

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V019_2017-07-10