



Large Volume Protein Labeling Kit RED-MALEIMIDE 2nd Generation

For Dianthus, Monolith NT.115 Series and NT.Automated Instruments with a RED
Detector

Cat# NT-L114

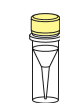
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1 About the kit

The Large Volume Protein Labeling Kit RED-MALEIMIDE 2nd Generation is optimized for labeling and purification of proteins with a molecular weight higher than 5 kDa. The dye carries a reactive maleimide group that reacts with cysteine residues to form a covalent bond. RED dyes are suited for Dianthus, Monolith NT.115 series and Monolith NT.Automated instruments with a RED detector (Nano and Pico). The kit contains material sufficient for 4 independent reactions.

Included in this kit



Dye RED-MALEIMIDE 2nd Generation (20 µg)
Carries a reactive maleimide group that reacts with cysteine residues to form a covalent bond.

4

-20 °C



Labeling Buffer MALEIMIDE
17.7 mM NaH₂PO₄, 32.5 mM Na₂HPO₄, 100 mM NaCl, pH 7 at room temperature

4

4 °C



A-Column
The A-Column provided can be used for the buffer exchange of 40-100 µL of a protein with a molecular weight higher than 5 kDa.

4

4 °C



B-Column Large
The B-Column provided can be used to purify proteins with a molecular weight higher than 5 kDa.

4

4 °C



Adapter (for 50 mL centrifuge tube)

2

RT



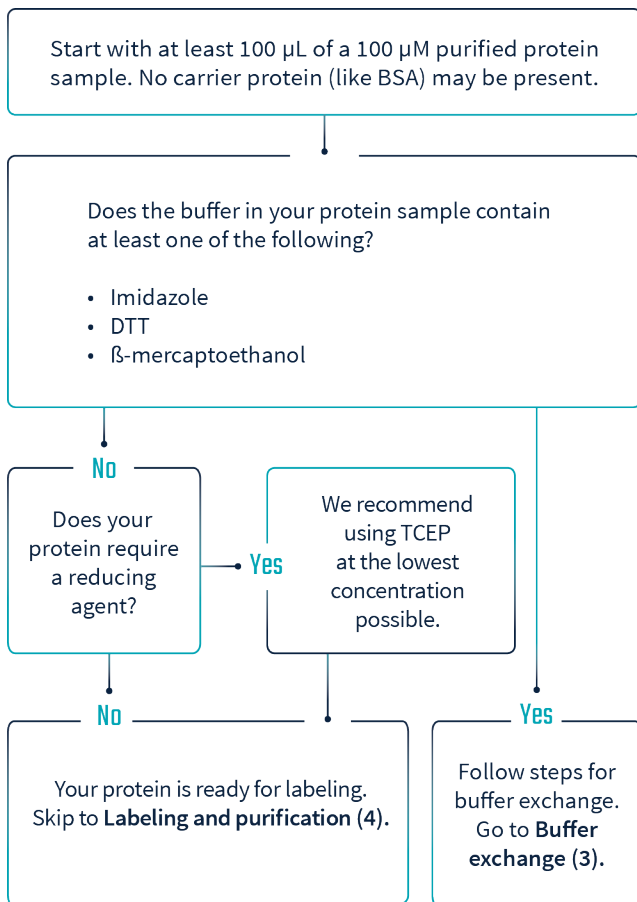
Bring all components to room temperature before use.
When stored appropriately, the kit components should be stable for approximately 12 months.
Do not exceed recommended centrifuge time or speed.



Need help? Visit the NanoTemper Explorer Community at nanotempertech.com/explore

2 Plan your labeling

Before you start protein labeling make sure that your sample is prepared in a compatible buffer. The decision tree below is intended to help you to determine if you need to perform an initial **Buffer exchange** or if you can directly start with **Labeling and purification**.



3 Buffer exchange

You will have to subject your protein sample to a buffer exchange if it's dissolved in an incompatible buffer. Your buffer cannot contain imidazole, DTT or β -mercaptoethanol and should be within pH 6.9-7.2.



If your protein is in a compatible buffer for labeling, skip this section and go straight to **Labeling and purification**.

Do not exceed recommended centrifuge time and speed.

To convert your protein concentration from mg/mL to μ M use the following formula:

$$\text{Concentration}(\mu\text{M}) = 1000 \times \frac{\text{Concentration}(\text{mg/mL})}{\text{MolecularWeight}(\text{kDa})}$$

Items you will need

Quantity



Labeling Buffer MALEIMIDE

1

Reconstitute with 3 mL of ddH₂O right before use. The final pH will be 7 at room temperature.

You will use only 1 mL in this step. The other 2 mL will be used for labeling your protein.



A-Column

1

The A-Column provided can be used for the buffer exchange of 40-100 μ L of a protein with a molecular weight higher than 5 kDa.

100 μ M highly pure protein sample

100 μ L

1.5 mL microcentrifuge tubes

2

Variable speed benchtop microcentrifuge (min. speed 1500 g)

1

Perform your buffer exchange



Invert the **A-Column** 3 times to resuspend slurry.



Twist off bottom and remove cap.



Place **A-Column** in a fresh microcentrifuge tube and centrifuge at 1500 g for 1 minute to remove storage solution.



Discard flow through from microcentrifuge tube and place **A-Column** back in the microcentrifuge tube.



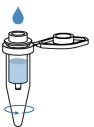
Add 300 μL **Labeling Buffer MALEIMIDE** to equilibrate the **A-Column**. Avoid contacting the inner walls of the column and load the buffer directly in the center of the resin bed.

Centrifuge at 1500 g for 1 minute and discard flow through.

Perform this step three times.



Place A-Column in a fresh microcentrifuge tube.



Pipette 100 μL of your 100 μM protein sample in the center of the column resin.

Centrifuge at 1500 g for 2 minutes.



Your protein is in the collected flow through.

The used column can be discarded.

4 Labeling and purification

Now you're ready to label and purify your protein.



Before you start with the labeling procedure, make sure your protein's concentration is ~20 μM . If it's significantly different, please see **Frequently asked questions** for suggestions.

Is your protein too concentrated? We recommend diluting it in the **Labeling Buffer MALEIMIDE** to ensure compatibility with the labeling protocol.

To convert your protein concentration from mg/mL to μM use the following formula:

$$\text{Concentration}(\mu\text{M}) = 1000 \times \frac{\text{Concentration}(\text{mg/mL})}{\text{MolecularWeight}(\text{kDa})}$$

Items you will need

Quantity



Dye RED-MALEIMIDE 2nd Generation (20 μg)

1

Reconstitute with 25 μL DMSO right before use by pipetting up and down. The final concentration will be 1060 μM .



Labeling Buffer MALEIMIDE

1

If you performed the buffer exchange, use the remaining 2 mL of reconstituted labeling buffer for this step.

Otherwise, reconstitute one vial with 3 mL of ddH₂O right before use. The final pH will be 7 at room temperature.



B-Column Large

1

The B-Column provided can be used to purify proteins with a molecular weight higher than 5 kDa.



Adapter (for 50 mL centrifuge tube)

1

1.5 mL microcentrifuge tubes

3

50 mL centrifuge tube

1

20 μM highly pure protein sample

450 μL

100 % DMSO

30 μL

Assay or equilibration buffer of choice

18 mL

Variable speed benchtop microcentrifuge (min. speed 1500 g)

1



Use gloves when handling DMSO and dye.

Use dye immediately after reconstitution.

Do not vortex your protein as it might compromise its integrity. Instead, gently pipette up and down to mix.

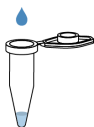


Do not exceed recommended incubation time.

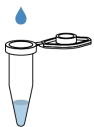
Use TCEP if a reducing agent is required during the labeling reaction.

Final DMSO concentration in the labeling reaction should not exceed 5%.

You're ready to start labeling



In a fresh microcentrifuge tube, mix 25 μL of **Dye RED-MALEIMIDE 2nd Generation** freshly prepared in DMSO with 25 μL **Labeling Buffer MALEIMIDE**. This gives you a 530 μM dye solution.

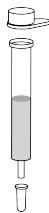


Add 50 μL of the 530 μM dye solution to 450 μL of your 20 μM protein sample in a microcentrifuge tube. Mix carefully by pipetting up and down several times. This yields 500 μL of dye-protein solution with an approximately 3-fold excess of dye.

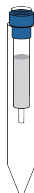


Incubate for 30 minutes at room temperature in the dark.

In the meantime: Equilibrate your B-Column Large (~ 20 minutes)



Remove top cap from the **B-Column Large** and pour off the storage solution. Then remove the bottom cap. Save both caps and set aside.



Replace the cap from the 50 mL centrifuge tube with the **Adapter**. Then place the column on top of the adapter and in the tube.



Fill column with assay or equilibration buffer of choice and allow buffer to enter the packed resin bed completely by gravity flow. Discard the flow through collected.

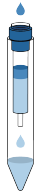
Repeat this step 3 more times.

About 15 mL of buffer should be used in total for all 4 steps. Discard the last flow through so the tube is ready for the next step.



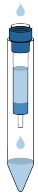
If you finish the equilibration before the 30 minutes labeling incubation is completed, place the caps you set aside back on the column to prevent the resin bed from drying out.

Purify your labeled protein by removing free dye



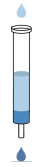
After the labeling reaction incubation, transfer the 500 μL of dye-protein solution to the B-Column Large you just equilibrated. Avoid contacting the inner walls of the column and load your sample directly in the center of the resin bed.

Let sample enter the resin bed completely.



Add 700 μL of assay or equilibration buffer of choice and allow buffer to enter the resin bed completely.

Have a fresh microcentrifuge tube ready to collect your labeled protein.



To elute your protein, place the fresh microcentrifuge tube under the column. Add 1200 μL of assay or equilibration buffer onto column and collect the flow through that contains your labeled protein. Avoid contacting the inner walls of the column and load your buffer directly in the center of the resin bed.

The used column can be discarded.



Most proteins can be stored after labeling for several weeks at $-80\text{ }^{\circ}\text{C}$ when aliquoted and flash-frozen in liquid nitrogen. Avoid freeze-thaw cycles.

5 Calculate concentration and degree-of-labeling (DOL)

We recommend to measure the concentration and the degree-of-labeling (DOL) of your labeled protein before you start your MST experiments. The DOL describes how many dye molecules are bound to your protein, e.g. a DOL of 1 refers to a dye:protein ratio of 1:1.



Measure absorption A_{280} and A_{650} .

If your protein contains no tyrosine or tryptophan residues, the absorption A_{205} , which arises primarily from the peptide bond, can be measured instead.

Depending on the sensitivity of your spectrophotometer and the concentration of your sample you may be out of detection range.

Typical path length d of a spectrophotometer is 1 cm.

Correction factor at 280 nm: 0.04

Correction factor at 205 nm: 0.19

Molar absorbance of dye: 195,000 M⁻¹cm⁻¹

Concentration determination at 280 nm

Provide the extinction coefficient $\epsilon_{\text{Protein}}$ of your protein.

Measure absorption at 280 nm and 650 nm.

Calculate concentration with following equation:

$$c(M) = \frac{A_{280} - (A_{650} \times 0.04)}{\epsilon_{\text{Protein}} \times d}$$

Concentration determination at 205 nm

Provide the molecular weight MW_{Protein} of your protein.

Measure absorption at 205 nm and 650 nm.

Calculate concentration with following equation:

$$c(M) = \frac{A_{205} - (A_{650} \times 0.19)}{31 \times MW_{\text{Protein(Da)}}$$

Calculate degree-of-labeling with following equation

$$DOL = \frac{A_{650}}{195,000 M^{-1} cm^{-1} \times c(M)}$$



Typical protein yield results are 50% to 70% after the purification step.

Optimal DOL is 0.5-1.

A DOL greater than 1 should be avoided for most proteins since it can lead to adverse effects on protein function.

A DOL below 0.5 should be avoided since it can lead to reduced signal-to-noise ratio. For more details see **Frequently asked questions**.

For the best result from your TRIC/MST assay, start with 10 nM of your RED-MALEIMIDE 2nd Generation labeled protein (Nano detector) or 5 nM (Pico detector), respectively.

Always use the latest version of DI.Control/MO.Control Software for optimal TRIC/MST assay planning.

6 Frequently asked questions

6.1 Can I use a different buffer from the Labeling Buffer MALEIMIDE?

The provided Labeling Buffer MALEIMIDE is the preferred buffer for the labeling reaction. It's a phosphate buffer with pH 7 at room temperature. However, you cannot use buffers that contain imidazole, DTT or β -mercaptoethanol. Use of other buffers may decrease labeling efficiency.

6.2 What adjustments should I make if the concentration of the protein sample I want to label is different than the recommended 20 μM ?

This table shows the adjustments you have to make to the dye solution if you want to label the protein at other concentrations. We do not recommend to label less than 2 μM of protein.



Reconstitute the dye in 25 μL DMSO to obtain a 1060 μM solution. Calculate the needed volumes of dye and buffer using the equations below.

Protein concentration (μM)	Dye excess	Dye concentration (μM)
2-5	5-fold	10-25
6-20	3-fold	18-60

Calculate the volume of Dye RED-MALEIMIDE 2nd Generation with following equation:

$$Volume_{Dye}(\mu L) = \frac{Concentration_{Protein}(\mu M) \times Dye_{excess}}{1060\mu M} \times 500\mu L$$

Calculate the volume of Labeling Buffer MALEIMIDE with following equation:

$$Volume_{Buffer}(\mu L) = 50\mu L - Volume_{Dye}(\mu L)$$



Mix Labeling Buffer MALEIMIDE with reconstituted RED-MALEIMIDE 2nd Generation dye as calculated above. Add 50 μL of this dye solution (100-600 μM) to 450 μL of your protein stock. Mix carefully by pipetting up and down several times. Incubate for 30 minutes at room temperature in the dark and continue with the standard protocol for **Labeling and purification**.

6.3 Can I increase the volume of my labeling reaction?

Yes, you can increase it up to 1200 μL , which is the maximum volume the provided B-Column can process. Adjust the volume to 1200 μL after the sample has entered the bed by using assay or equilibration buffer.

6.4 The calculated DOL is significantly higher than recommended. What could be the reason?

If your calculated DOL is significantly higher than recommended, your protein is most likely over-labeled. Reduce the ratio of dye to protein.

6.5 The calculated DOL is significantly less than recommended. What could be the reason?

If your calculated DOL is significantly less than recommended, your protein is most likely under-labeled. Here is a list of potential reasons and proposed solutions:

Reason	Explanation	Solution
The buffer contains free sulfhydryls.	Free sulfhydryls (like DTT or protein impurities) present in the buffer will react with the labeling dye and decrease the labeling efficiency.	Perform the buffer exchange prior to the labeling and consider to replace DTT with TCEP for labeling. TCEP should be removed on the B-Column Large after labeling.
Protein purity is too low.	The presence of carrier proteins like BSA or casein will interfere with the labeling and subsequent TRIC/MST assay.	Use the protein of appropriate purity.
pH of the labeling reaction is not optimal.	The maleimide reaction is most efficient near neutral pH (pH 6.9 to 7.2).	Perform the buffer exchange prior to the labeling.
Protein concentration is too low.	An optimal ratio between protein and dye is required for successful labeling.	Concentrate your protein by using spin concentrators.

7 Notes

8 Safety information

RED-MALEIMIDE 2nd Generation



Hazard statements

- H302 Harmful if swallowed.
H318 Causes serious eye damage.
H335 May cause respiratory irritation.



Precaution statements

- P261 Avoid breathing dust/fume/gas/mist/vapors/spray.
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.
P403+P233 Store in a well-ventilated place. Keep container tightly closed.
P501 Dispose of waste according to applicable legislation.

B-Column Large



Hazard statements

- H317 May cause an allergic skin reaction.



Precaution statements

- P261 Avoid breathing dust/fume/gas/mist/vapors/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.

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

9 Purchaser notification

NanoTemper grants the buyer the non-transferable right to use the purchased product for research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components for commercial purposes.

Limited warranty.

NanoTemper will replace any product that does not meet the specifications. This warranty limits NanoTemper's liability only to the cost of the product when within the expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. NanoTemper assumes no responsibility or liability for any indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive.

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