Protein Labeling Kit RED-NHS 2nd Generation

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

Cat# MO-L011

Content

1 About the kit

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

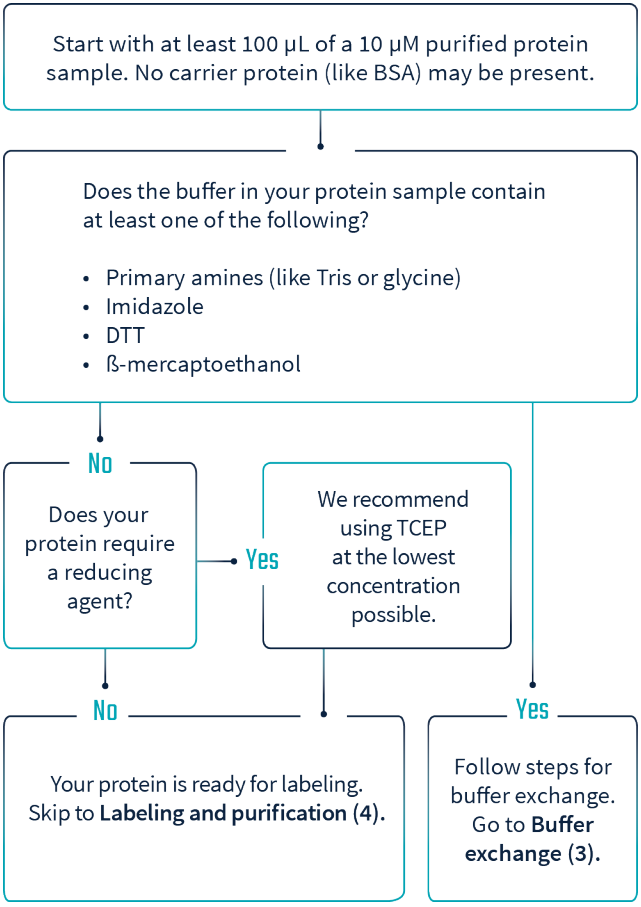
Included in this kit	Quantity	Storage
 **Dye RED-NHS 2nd Generation (10 µg)** Carries a reactive NHS-ester group that reacts with primary amines to form a covalent bond.	4	-20 °C
 **Labeling Buffer NHS** 130 mM $NaHCO_3$, 50 mM NaCl, pH 8.2-8.3 at room temperature	4	4 °C
 **A-Column** The A-Column provided 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** The B-Column provided can be used to purify proteins with a molecular weight higher than 5 kDa.	4	4 °C
 Adapter (for 15 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 primary amines (like Tris or glycine), imidazole, DTT or β-mercaptoethanol and should be within pH 8.2-8.5.

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 µM use the following formula:

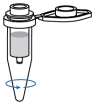
$$Concentration(\mu M) = 1000 \times \frac{Concentration(mg/mL)}{MolecularWeight(kDa)}$$

Items you will need	Quantity
Labeling Buffer NHS Reconstitute with 3 mL of ddH_2O right before use. The final pH will be 8.2-8.3 at room temperature. You will use only 1 mL in this step. The other 2 mL will be used for labeling your protein.	1
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.	1
10 µM highly pure protein sample	100 µL
1.5 mL microcentrifuge tubes	2
Varibable speed benchtop microcentrifuge (min. speed 1500 g)	1

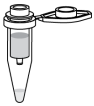
Perform your buffer exchange

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 NHS** 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 10 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 ~10 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 NHS** to ensure compatibility with the labeling protocol.

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

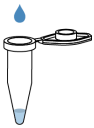
$$Concentration(\mu M) = 1000 \times \frac{Concentration(mg/mL)}{MolecularWeight(kDa)}$$

Items you will need	Quantity
 **Dye RED-NHS 2nd Generation (10 µg)** Reconstitute with 25 µL DMSO right before use by pipetting up and down. The final concentration will be 600 µM.	1
 **Labeling Buffer NHS** 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 ddH_2O right before use. The final pH will be 8.2-8.3 at room temperature.	1
 **B-Column** The B-Column provided can be used to purify proteins with a molecular weight higher than 5 kDa.	1
 Adapter (for 15 mL centrifuge tube)	1
1.5 mL microcentrifuge tubes	3
15 mL centrifuge tube	1
10 µM highly pure protein sample	90 µL
100 % DMSO	30 µL
Assay or equilibration buffer of choice	12 mL
Variable speed benchtop microcentrifuge (min. speed 1500 g)	1

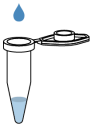
Use gloves when handling DMSO and dye.
Use dye immediately after reconstitution.
Do not vortex your protein as it might compromise it's integrity. Instead, gently pipette up and down to mix.

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 7 µL of **Dye RED-NHS 2nd Generation** freshly prepared in DMSO with 7 µL **Labeling Buffer NHS**. This gives you a 300 µM dye solution.

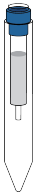


Add 10 µL of the 300 µM dye solution to 90 µL of your 10 µM protein sample in a microcentrifuge tube. Mix carefully by pipetting up and down several times. This yields 100 µL of dye-protein solution with an approximately 3-fold excess of dye.

Incubate for 30 minutes at room temperature in the dark.

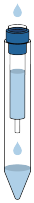
In the meantime: Equilibrate your B-Column (~ 20 minutes)

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



Replace the cap from the 15 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.

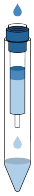
Discard the flow through collected.

Repeat this step 3 more times.

About 8-10 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.

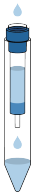
If you finish the equilibration before the 30 minutes labeling incubation is completed, place the caps you set aside back on the column to prevent the resin bed from drying out.

Purify your labeled protein by removing free dye



After the labeling reaction incubation, transfer the 100 µL of dye-protein solution to the B-Column 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 550 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 450 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 °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 binding 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 $M^{-1}cm^{-1}$

Concentration determination at 280 nm

Provide the extinction coefficient $\varepsilon_{Protein}$ of your protein.
Measure absorption at 280 nm and 650 nm.
Calculate concentration with following equation:

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

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:

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

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 assay, start with 20 nM of your RED-NHS2nd Generation labeled protein (Nano detector) or 5 nM (Pico detector), respectively.

Always use the latest version of Control Software for optimal assay planning.

6 Frequently asked questions

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

The provided Labeling Buffer NHS is the preferred buffer for the labeling reaction. It's a carbonate buffer with pH 8.2-8.3 at room temperature. However, you cannot use buffers that contain primary amines (like Tris or glycine), imidazole, DTT or β-mercaptoethanol. Use of other buffers may decrease labeling efficiency. If you want to achieve more specific labeling of the amine terminus, use a buffer with closer to neutral pH, as the pK_a of the terminal amine is lower than that of the lysine ε-amino group.

6.2 What adjustments should I make if the concentration of the protein sample I want to label is different than the recommended 10 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 600 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
6-10	3-fold	18-30
11-20	2-fold	22-40

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

$$Volume_{Dye}(\mu L) = \frac{Concentration_{Protein}(\mu M) \times Dye_{excess}}{600 \mu M} \times 100 \mu L$$

Calculate the volume of Labeling Buffer NHS with following equation:

$$Volume_{Buffer}(\mu L) = 10 \mu L - Volume_{Dye}(\mu L)$$

Mix Labeling Buffer NHS with reconstituted RED-NHS 2nd Generation dye as calculated above.
Add 10 µL of this dye solution (100-400 µM) to 90 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 prepare up to 500 µL labeling reaction and transfer it to the B-Column (500 µL is the maximum volume the provided B-Column can process). Let the sample enter the resin bed completely, before adding assay or equilibration buffer to adjust the volume to 650 µL. Let the buffer enter the resin bed and elute the labeled protein according to the instructions described in step 4 above.

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
NHS ester is non-reactive.	NHS esters have a half-life of only 10 minutes at pH 8.6 and about an hour at pH 8.	Use the diluted dye solution immediately after preparing it.
The buffer contains primary amines.	Primary amines (like Tris or glycine) present in the buffer will react with the labeling dye and decrease the labeling efficiency.	Perform the buffer exchange prior to the labeling.
Protein purity is too low.	The presence of carrier proteins like BSA or casein will interfere with the labeling and subsequent binding assay.	Use the protein of appropriate purity.
pH of the labeling reaction is not optimal.	NHS esters react most efficiently with primary amines in physiological to slightly alkaline conditions (pH 7.2 to 9).	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-NHS 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

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.

How can I place an order?

Shopping in the US? Use our online webshop at
shop.nanotempertech.com

From all other countries, request a quote online here
nanotempertech.com/purchase

NanoTemper Technologies GmbH | Flössergasse 4, 81369 München

Phone +49 (0) 89 4522895 0 | Fax +49 (0) 89 4522895 60

info@nanotempertech.com | nanotempertech.com