SNAP-Tag® Labeling Kit RED 2nd Generation

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

Cat# MO-L019

Content

1 About the kit

The SNAP-Tag® Labeling Kit RED 2nd Generation is optimized for labeling and purification of proteins co-expressed with a N- or C-terminal SNAP-Tag®. The dye carries a Benzylguanine group that reacts with the SNAP-Tag 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.

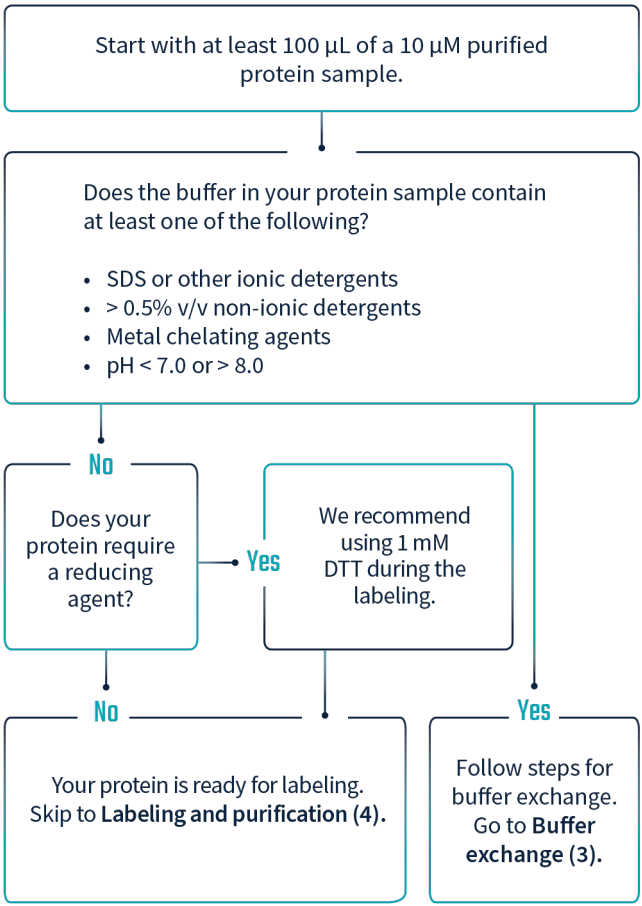
Included in this kit	Quantity	Storage
 **Dye RED-SNAP-Tag® 2nd Generation (10 nmol)** Carries a Benzylguanine-group that reacts with the SNAP-Tag® to form a covalent bond.	1	-20 °C
 **A-Column** The A-Column provided can be used for the buffer exchange of 40-100 µL of a protein with a molecular weight 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 15mL centrifuge tube)	2	RT

Bring all components to room temperature before use.
When stored appropriately, the kit components should be stable for approximately 6 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 SDS or other ionic detergents, metal chelating agents (e.g. EDTA or EGTA) or more than 0.5 % v/v non-ionic detergents and should be within pH 7.0 - 8.0. Generally, your fusion partner should dictate your buffer composition, contain 50 - 250 mM monovalent salts and be within pH 7.0 - 8.0. Addition of 1 mM DTT may enhance your labeling efficiency as it improves the stability and reactivity of the SNAP-tagged protein.

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

Do not exceed recommended centrifuge time and speed.

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

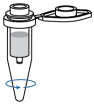
$$Concentration(\mu M) = 1000 \times \frac{Concentration(mg/mL)}{MolecularWeight(kDa)}$$

Items you will need	Quantity
 **Labeling Buffer of choice (not provided)** Make sure your buffer is within pH 7.0 - 8.0 and does not contain metal chelating agents, SDS or other ionic detergents and less than 0.5 % v/v non-ionic detergents. We recommend PBS buffer (supplemented with 0.05 % Tween-20). Addition of 1 mM DTT may enhancce your labeling efficiency.	1 mL
 **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

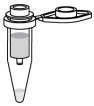
Perform your buffer exchange

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** 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.

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

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

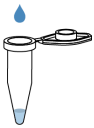
$$Concentration(\mu M) = 1000 \times \frac{Concentration(mg/mL)}{MolecularWeight(kDa)}$$

Items you will need		Quantity
	**Dye RED-SNAP-Tag® 2nd Generation (10 nmol)** Reconstitute with 25 µL DMSO right before use by pipetting up and down. The final concentration will be 400 µM.	1
	**Labeling Buffer of choice (not provided)** Make sure your buffer is within pH 7.0 - 8.0 and does not contain metal chelating agents, SDS or other ionic detergents and less than 0.5 % v/v non-ionic detergents. We recommend PBS buffer (supplemented with 0.05 % Tween-20). Addition of 1 mM DTT may enhancce your labeling efficiency.	1 mL
	**B-Column** The B-Column provided can be used to purify proteins with a molecular weight higher than 5 kDa.	1
	Adapter (for 15mL 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

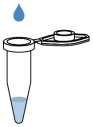
Use gloves when handling DMSO and dye.
Do not vortex your protein as it might compromise it's integrity. Instead, gently pipette up and down to mix.

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 6 µL of RED-SNAP-Tag® 2nd Generation in DMSO with 6 µL **Labeling Buffer of choice**. This gives you a 200 µM dye solution.

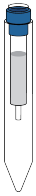


Add 10 µL of the 200 µM dye solution to 90 µL of your 10 µM protein sample in a microcentrifuge tube. Mix carefully by pipetting up and down several times. This yields 100 µL of dye-protein solution with an approximately 2-fold excess of dye.

Incubate for 30 minutes at room temperature in the dark.

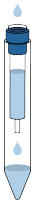
In the meantime: Equilibrate your B-Column (~ 20 minutes)

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.

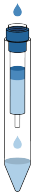
Discard the flow through collected.

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.

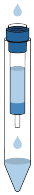
If you finish the equilibration before the 30 minutes labeling incubation is completed, place the caps you set aside back on the column to prevent the resin bed from drying out.

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.

Dissolved dye is stable at -20 °C for 3 month.

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: 0.06

Correction factor at 205 nm: 0.42

Molar absorbance of dye: 220,000 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:

$$c(M) = \frac{A_{280} - (A_{650} \times 0.06)}{\epsilon_{Protein} \times d}$$

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:

$$c(M) = \frac{A_{205} - (A_{650} \times 0.42)}{31 \times MW_{Protein(Da)}}$$

Calculate degree-of-labeling with following equation

$$DOL = \frac{A_{650}}{220,000 M^{-1} cm^{-1} \times c(M)}$$

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

Optimal DOL is 0.5-1.

For the best result from your TRIC/MST assay, start with 20 nM of your RED-SNAP-Tag® 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 increase the volume of my labeling reaction?

Yes, you can increase it up to 500 µL, which is the maximum volume the provided B-Column can process. Adjust the volume to 650 µL after the sample has entered the bed by using assay or equilibration buffer.

6.2 The calculated DOL is significantly less than recommended. What can I do?

You can enhance the stability and reactivity of the SNAP-tag® protein by adding 1 mM DTT to your labeling buffer. Prolonging the labeling reaction time to 1 hour may also increase your DOL.

7 Safety information

RED-SNAP-Tag® 2nd Generation

Hazard statements

H318	Causes serious eye damage.

Precaution 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.

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.

8 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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