Best Practices for Protein Labeling

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

Start off right with your MST assay development

Assay development for MST begins with protein labeling. NanoTemper Technologies provides several labeling strategies and fluorophores optimized for high data quality in your MST assay.

In the following you will find our **best practice** for protein labeling, recommended **Monolith labeling kits**, a **labeling decision tree** that will help you to find the best labeling strategy for your specific protein and helpful **recommendations** on how to determine the efficiency of labeling, how to deal with difficult proteins and how to troubleshoot your MST assay.

Best practice

Recommended: RED-tris-NTA labeling

We recommend to express or buy your protein with a His-Tag and to use the His-Tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018).

This kit offers a versatile tool for efficient and site-specific *in situ* labeling of His-tagged proteins. The labeling procedure is robust towards a variety of different buffer conditions and components, plus the high affinity and selectivity of the dye for His-Tags enables the labeling of target proteins even in crude cell lysates.

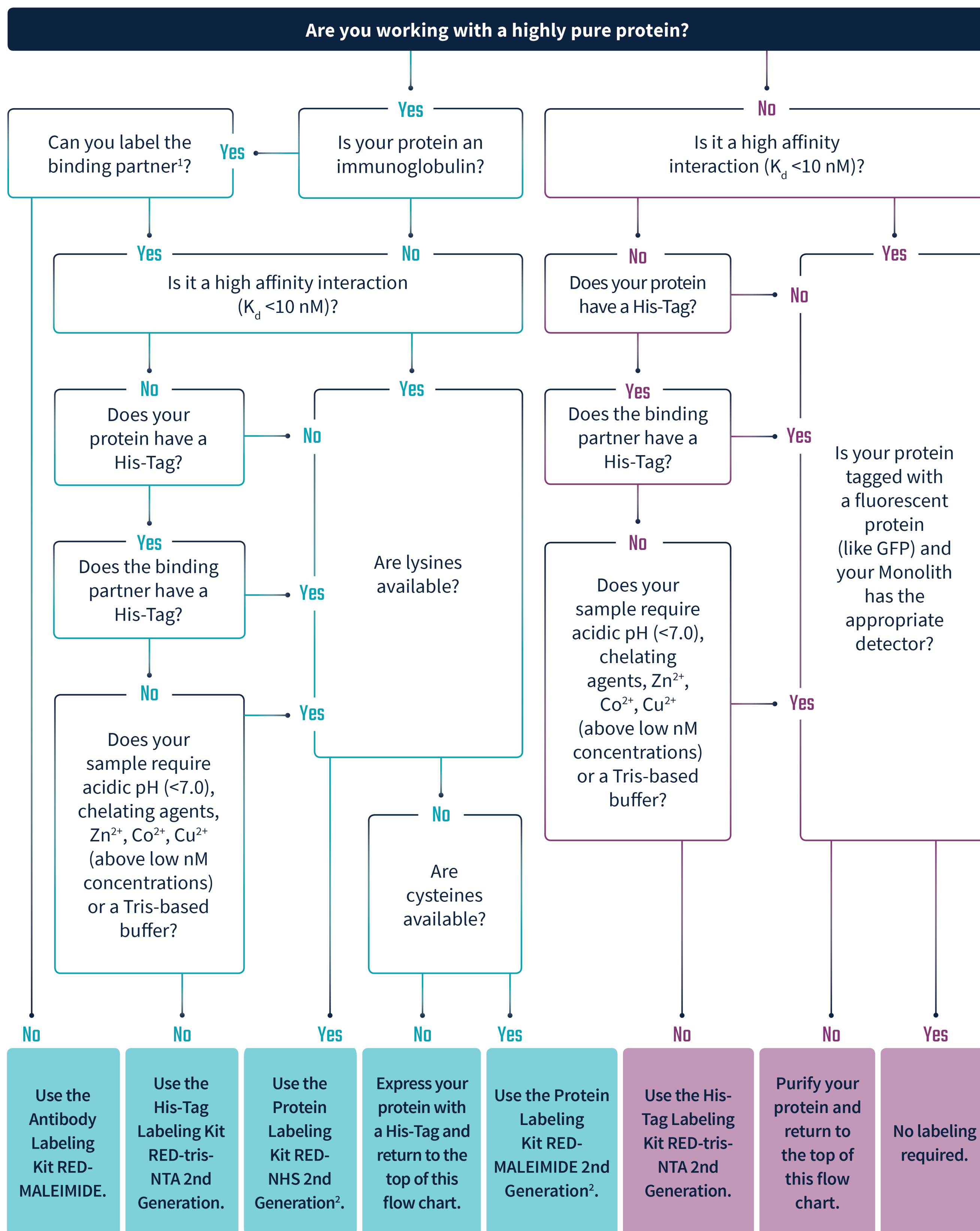
Monolith labeling kits

Recommended labeling kits provided by NanoTemper Technologies:

Catalog number	Product
MO-L018	**His-Tag Labeling Kit RED-tris-NTA 2nd Generation** The kit is used for site-specific and purification-free labeling of His-tagged (6x His) proteins and peptides and can be used with purified as well as crude samples (e.g. cell lysate). Contains material sufficient for 500 single-point MST measurements.
MO-L011	**Protein Labeling Kit RED-NHS 2nd Generation** The kit utilizes a dye carrying a reactive NHS-ester group that reacts with primary amines (lysine residues) to form a covalent bond. The kit is optimized for proteins with a molecular weight higher than 5 kDa and concentrations between 2 and 20 µM. Contains material for 4 independent reactions.
MO-L014	**Protein Labeling Kit RED-MALEIMIDE 2nd Generation** The kit utilizes a dye carrying a reactive maleimide group that reacts with cysteine residues to form a covalent bond. The kit is optimized for proteins with a molecular weight higher than 5 kDa and concentrations between 2 and 20 µM. Contains material for 4 independent reactions.
MO-L007	**Antibody Labeling Kit RED-MALEIMIDE** The kit enables site-specific labeling of antibodies by selective reduction of disulfide bonds and subsequent labeling of free cysteins. Contains material for 4 independent reactions.

Which labeling kit should I use?

The labeling decision tree below is intended to assist you in finding the best labeling strategy for your target protein.



¹ *we recommend to label the binding partner when working with immunglobulins*

² *suitable for proteins larger than 5 kDa*

Was my labeling reaction successful?

Before starting your MST experiment, test the efficacy of your protein labeling reaction by utilizing the appropriate strategy below for your protein labeling kit of choice.

RED-tris-NTA labeling

After the protein labeling procedure, use the pretest routine in the MO.Control software to check whether the dye was properly dissolved. At 50 nM of labeled protein, we expect to measure 300 fluorescence counts at a LED power of 40 % on the Monolith NT.115, and 12,000 fluorescence counts for 10 nM of protein on the Monolith NT.115 Pico, following the exact recommendations of the His-Tag labeling protocol.

Note: Prior to labeling, we recommend performing a binding experiment between the His-tagged protein and the RED-tris-NTA dye to determine the affinity between the dye and your protein. You may follow the instructions of the kit. We want the protein:dye ratio such that the dye should be bound and not have any free dye in solution.

NHS- or Maleimide labeling

Determine the degree of labeling

The degree of labeling (DOL) specifies the number of dye molecules attached to one protein molecule. We recommend a DOL of 0.5 to 1. To calculate the DOL, use a UV/VIS spectrophotometer to measure the absorbance spectrum of your sample and then use the following equation to calculate the DOL.

$$DOL = \frac{c_{Dye}}{c_{Protein}}$$

For detailed instructions on the determination of protein concentration and DOL please refer to our FAQs, the Nanopedia in the MO.Control software or contact your local Application Specialist.

Protein quality check - a comparison of labeled and unlabeled protein

Evaluate protein quality before and after labeling to ensure that your protein remains properly folded after covalent modification with a fluorophore. This can be done by comparing structural integrity data of labeled and unlabeled protein using an instrument like the Tycho NT.6.

What can I do if my labeling reaction was not successful?

DOL too low

- Check the buffer for additives unsuitable for the labeling reaction (see labeling manuals).
- For NHS- and Maleimide labeling: Increase the dye:protein ratio to 5:1 or 7:1. Make sure that the DMSO concentration will not exceed 5 % in the labeling mixture.
- Make sure that the dye was properly dissolved.

DOL too high

- For NHS- and Maleimide labelign: Decrease the dye:protein ratio to 2:1 or 1:1.
- Incubate the labeling reaction on ice.

Tycho measurements show no unfolding transition for my labeled protein

- Decrease the dye:protein ratio.
- Make sure that the protein tolerates the labeling buffer and the labeling conditions.
- Use the RED-tris-NTA 2nd Generation Labeling Kit for a gentler protein labeling reaction.

My labeling was successful, but I cannot get a binding curve.

Test different buffers and buffer additives

If the signal-to-noise ratio is too low to conclude binding, we recommend testing different buffers (e.g. PBS-, HEPES- or Tris-based buffers). Additionally, double-check that the buffer contains all necessary additives for the interaction being studied, such as reducing agents, cofactors and so on.

If DMSO is necessary in the assay buffer, test that the target protein can tolerate the used DMSO concentration. This can be done by evaluating the stability of the protein in assay buffer with varying DMSO concentrations, quickly done on the Tycho NT.6 in just 3 minutes.

Change the labeling strategy

If your protein was labeled with the NHS- or Maleimide labeling kits, consider the possibility that lysine or cysteine residues located in the protein's active site and involved in the binding of protein to ligand may have been compromised by the labeling reaction. As a solution, change your protein labeling strategy to site-specific labeling of recombinant His-Tags with the RED-tris-NTA 2nd Generation dye. Another option is to protect the active site with a low-affinity ligand prior to the covalent protein labeling strategy. After the protein is covalently labeled, the bound ligand can be removed using size-exclusion chromatography.