Cat# NT-L020

Biotinylated Target Labeling Kit

For Monolith and Dianthus Instruments with Spectral shift

Content and Storage

Biotinylated Target Labeling Kit is shipped at room temperature.

Each kit contains material sufficient for 500 single-point spectral shift measurements.

1* 20 pmol labeling dye (50 µL, 400 nM)

Store at -20 °C

Expiration date: see kit box label

Additional Material Required

- 1.5 mL microcentrifuge collection tubes
- 384 microwell plates or 0.2 mL PCR tubes
- Buffer of choice

Labeling Procedure

The Biotinylated Target Labeling Kit provides convenient means for the site-specific, purification-free labeling of small amounts of biotinylated molecules (e.g., proteins) with our fluorescent dye. This kit can be used for the labeling of any protein or peptide carrying one or multiple biotin-tags and contains material sufficient for labeling for 500 single-point spectral shift measurements. Labeling can be completed in 15 minutes; no removal of excess dye is required. The labeling dye binds efficiently to biotin-tags and shows fluorescence excitation and emission maxima at approximately 650 nm and 670 nm, respectively.

Important Information Before Starting

Biotin-tags are common protein tags which are routinely used for example for immobilization on surfaces coated with streptavidin. The biotin-tag labeling strategy is highly specific, requires only nanomolar (nM) concentrations of biotinylated proteins and no dye-removal step. Labeling can be carried out even with unpurified samples, in cell lysate or other complex bioliquids. Moreover, biotin-tag labeling is robust towards a variety of common storage and assay buffer components. Complexes are also extremely stable over a wide range of temperature and pH. We recommend using phosphate-buffered saline (PBS) supplemented with 0.005 % Tween 20 or alternatively HEPES buffer and a pH in the range of 7-8 for the labeling reaction. To ensure a high labeling efficiency, we recommend to initially determine the affinity between the dye and the biotinylated protein of interest (Step A).

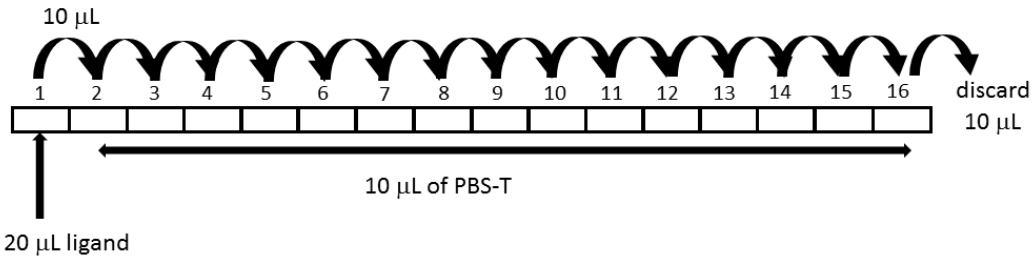
Component	***Maximum allowed/tested concentration***
General	
Buffer System	Tris, PBS, HEPES
pH	5 – 8
NaCl	Up to 800 mM
$MgCl_2$	Up to 200 mM
Reducing Agents	
DTT	10 mM
TCEP	4 mM
GSH	80 mM
Detergents/Chaotropes	
Tween-20	0.05 %
Pluronic F-127	0.01 %
SDS	Not recommended
Urea	Not recommended
Guanidinium Hydrochloride	Not recommended
Nucleotides	
AMP/ADP/ATP	8 mM
GMP/GDP/GTP	4 mM
Excipients	
EDTA	0.4 mM
Biotin	Not recommended
Glycerol	Up to 5 %
PEG 8,000	0.1 %
DMSO	5.0 % (lowers S/N)
Imidazole	Up to 50 mM (lowers S/N)

Step A
Affinity of dye to target Molecule

What is the affinity of the labeling dye to the biotinylated molecule of interest?

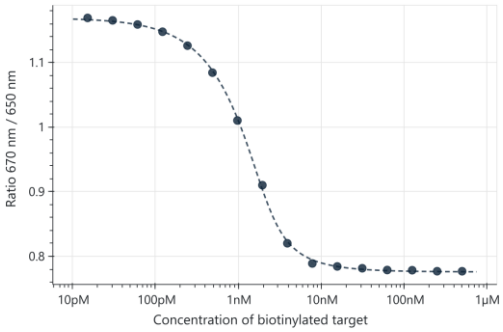
To determine the affinity of the labeling dye for the biotinylated molecule of interest or to determine the labeling efficiency of the molecule in the final experimental interaction buffer, the following experimental procedure is recommended. PBS-T (0.005 % Tween 20) buffer can be replaced by a different assay buffer, if required by the user.

1. Unpack the kit and thaw the labeling dye vial. Spin the vial for a few second to ensure that any liquid stuck to the cap is spun to the bottom the vial and not lost upon opening.
2. Prepare 200 µL of a 4 nM solution of the labeling dye in PBS-T by mixing 2 µL of dye (400 nM) and 198 µL PBS-T.
3. Prepare 25 µL of 1 µM biotinylated molecule in PBS-T.
4. Transfer 10 µL of PBS-T into wells/PCR-tubes **2-16**.
5. Transfer 20 µL of 1 µM biotinylated molecule solution into the first well/PCR-tube.
6. Transfer 10 µL of the ligand from well/PCR-tube **1** to well/PCR-tube **2** with a pipette and mix by pipetting up-and-down multiple times. Transfer 10 µL to well/PCR-tube **3** and mix. Repeat the procedure for wells/PCR-tubes **4-16**. Discard the extra 10 µL from well/PCR-tube 16.



7. Add 10 µL of 4 nM labeling dye solution to each well/PCR-tube **in reverse order** (16-1) and mix by pipetting. If you are using a Dianthus, spin the microwell plate.
8. Incubate for 15 min at room temperature.
9. Measure the samples in the Dianthus using auto-excitation or load the capillaries and measure the samples at 100 % LED/excitation power in the Monolith.
10. The K_d can be determined in DI.Control/MO.Control or DI.Screening Analysis using the K_d fit.

The interaction between labeling dye and biotinylated molecules is usually very strong, so the dose response curve may display a so-called saturation kink at ~2 nM and a fitted K_d below 200 pM:



Please note:

For some biotinylated targets, the accessibility of the biotin could be restricted, which will result in a larger K_d. Also, the binding kinetics to the labeling can be very slow, requiring longer incubation times. If the affinity between the labeling dye and your biotinylated molecule of interest is too low even after long incubation (K_d > 20 nM), we recommend switching to a covalent labeling strategy for lysine (Cat. # MO-L011) or cysteine (Cat. # MO-L014) residues.

STEP B MOLECULE LABELING

The following protocol describes the labeling procedure for one experiment with 16 microwells/capillaries. PBS-T (0.005 % Tween 20) buffer can be replaced by a different assay buffer, if required by the user*. Volumes can be scaled up- or down as necessary.*

1. Prepare an 8 nM dye solution by mixing 2 µL of labeling dye (400 nM) and 98 µL PBS-T.
2. Adjust the protein concentration to 80 nM in a volume of 100 µL.
   (If the K_d obtained in Step A is larger than 4 nM, use **at least 20x the obtained K_d concentration**.)
3. Mix 90 µL of protein (80 nM) with 90 µL of dye (8 nM).
4. Incubate for 15 minutes at room temperature.
5. The molecule is labeled and ready for the binding assay.

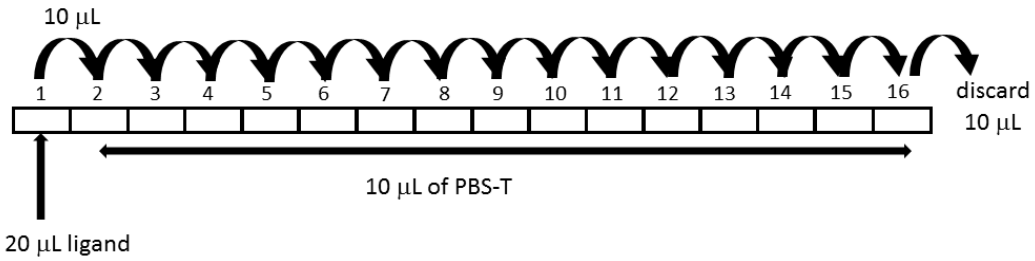
Please note:

For high affinity interactions, the usage of a higher protein concentration can influence your K_d determination (if the protein concentration in the assay is a lot above the K_d of your interaction you can only determine an EC50). If the affinity between labeling dye and your molecule of interest is too low (K_d > 20 nM) we recommend adjusting your assay buffer to improve the affinity or switching to a covalent labeling strategy for lysine (Cat. # NT-L011) or cysteine (Cat. # NT-L014) residues.

STEP C
BINDING ASSAY

We recommend preparation of serial dilutions in PCR tubes or in 384-well multi-well plates with a non-binding surface. PBS-T (0.005 % Tween 20) buffer can be replaced by a different assay buffer, if required by the user.

1. Prepare 25 µL of the ligand at 2 x concentration in PBS-T or assay buffer of choice (e.g., for a final concentration of 500 nM, prepare ligand at a concentration of 1000 nM). Make sure to avoid buffer mismatches within your titration series.
2. Add 10 µL of PBS-T into the wells/PCR-tubes **2-16**.
3. Transfer 20 µL of the ligand into well/PCR-tube **1**.
4. Transfer 10 µL of the ligand from well/PCR-tube **1** to well/PCR-tube **2** with a pipette and mix by pipetting up-and-down multiple times. Transfer 10 µL to well/PCR-tube **3** and mix. Repeat the procedure for well/PCR-tube **4-16**. Discard the extra 10 µL from well/PCR-tube **16**.



5. Add 10 µL of labeled biotinylated molecule to each well/PCR-tube in reverse order (16-1) and mix by pipetting. The final concentration of biotinylated target in the assay is 20 nM. This concentration should be used for the calculation of the K_d value. If you are using a Dianthus, spin the microwell plate and then seal the plate with sealing foil.
6. Measure your samples in the Dianthus using auto-excitation or load the capillaries and measure the samples at 100 % LED/excitation power in the Monolith. At the final dye concentration of 2 nM the expected fluorescence intensity at 100 % LED is around 400 counts on a Monolith X.

FAQ

1. *The signal-to-noise ratio of my experiment is too poor to allow data analysis. How can I improve the ratio?*

   Free dye in the solution might impair the signal-to-noise ratio. In case the concentration of protein prior to labeling has been overestimated, excess dye may be present. We recommend to re-check the concentration of your protein or to increase the ratio between the protein and the dye.

2. *Can I aliquot the labeling dye solution?*

   Yes, we recommend aliquoting out the dye solution in multiple aliquots and storing the aliquots at -20 °C to prevent multiple freeze-thaws.

3. *Can I use labeling dye to label unpurified biotinylated protein in cell lysates?*

   Yes, labeling unpurified biotin-tagged protein with labeling dye is possible if the dye retains strong affinity towards the biotin-tagged protein in cell lysate. This should be tested prior to performing the binding assay (Step A, page 3). Strong detergents often present in lysis buffers should be avoided (e.g., SDS) since they tend to disrupt binding interactions. It is recommended to use mechanical force (e.g., Dounce homogenizer) and PBS buffer for cell homogenization. In complex environments like cell lysate, the use of appropriate controls is always advisable to control for unspecific interactions.

SAFETY INFORMATION

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

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

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