Cat# NT-L130

Large Volume Human Fc Labeling Kit

For Monolith and Dianthus Instruments with Spectral Shift

Content and Storage

Large Volume Human Fc Labeling Kit is shipped at room temperature.

Each kit contains material sufficient for 4000 single-point spectral shift measurements.

1* 400 pmol Labeling dye (80 µL, 5 µM) in 100% DMSO

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 Large Volume Human Fc Labeling Kit provides convenient means for the site-specific, purification-free labeling of IgG antibodies with a human Fc region with our fluorescent dye. This kit contains material sufficient for labeling for 4000 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 human Fc regions and shows fluorescence excitation and emission maxima at approximately 650 nm and 670 nm, respectively.

Important Information Before Starting

The Human Fc labeling strategy is highly specific, requires only nanomolar (nM) concentrations of human IgG antibodies and has no dye-removal step. Labeling can be carried out even with unpurified samples, in cell lysate or other complex bioliquids. Moreover, Human Fc 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 antibody of interest (Step A).

See below for a supplemental list of already tested commonly used additives and their maximum allowed concentrations for buffer optimization.

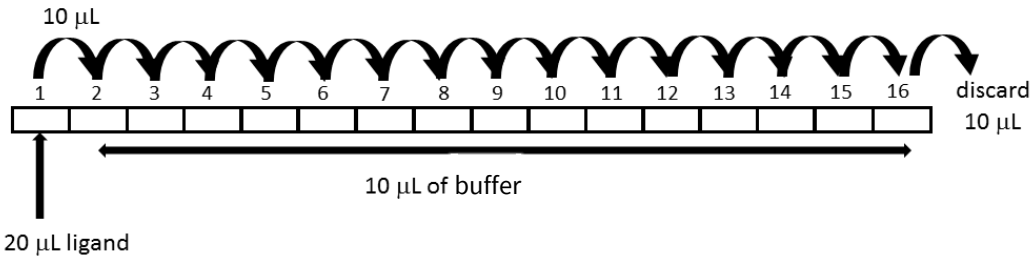
Component	***Maximum allowed/tested concentration***
General	
Buffer System	PBS, HEPES, Tris
pH	5 – 8
NaCl	Up to 300 mM
$MgCl_2$	Up to 200 mM
Reducing Agents	
DTT	Not recommended
TCEP	Not recommended
GSH	Up to 80 mM
Detergents/Chaotropes	
Tween 20	0.05 %
Pluronic F-127	0.05 %
SDS	Not recommended
Urea	Not recommended
Guanidinium Hydrochloride	Not recommended
Nucleotides	
AMP/ADP/ATP	8 mM
GMP/GDP/GTP	4 mM
Excipients	
EDTA	0.4 mM
Biotin	8 mM
Glycerol	Up to 40 %
Imidazole	Up to 50 mM
BSA	Up to 100 nM
Protein A	Not recommended

Step A
Affinity of dye to target Molecule

What is the affinity of the labeling dye to the Fc region of antibodies?

To determine the affinity of the labeling dye for antibodies or to determine the labeling efficiency of the antibodies 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.
2. Spin the vial for a few seconds to ensure that any liquid stuck to the cap is spun to the bottom the vial and not lost upon opening.
3. Prepare 1000 µL of a 10 nM solution of the labeling dye in buffer by mixing 2 µL of dye (5 µM) and 998 µL buffer.
4. Prepare 25 µL of 1 µM antibody in buffer.
5. Transfer 10 µL of buffer into wells/PCR-tubes **2-16**.
6. Transfer 20 µL of 1 µM antibody solution into the first well/PCR-tube.
7. 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.



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

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 20 nM dye solution by mixing 2 µL of labeling dye (5 µM) and 498 µL buffer.
2. Adjust the antibody concentration to 80 nM in a volume of 100 µL.
   (If the K_d obtained in Step A is larger than 2 nM, use **at least 20x the obtained K_d concentration**.)
3. Mix 90 µL of antibody (80 nM) with 90 µL of dye (20 nM).
4. Incubate for 15 minutes at room temperature.
5. The antibody is labeled and ready for the binding assay.

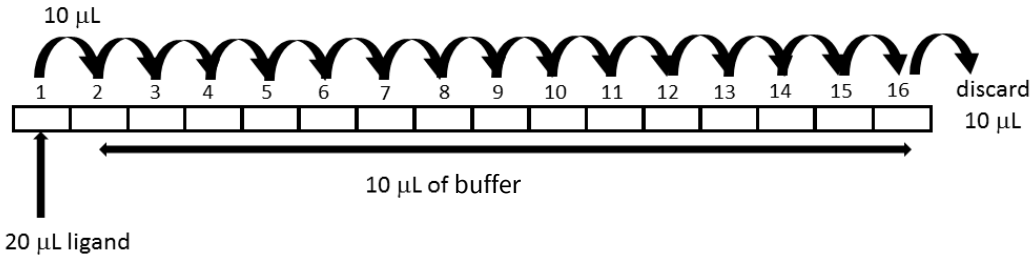
Please note:

An accurate Kd determination according to the law of mass action requires that the target concentration in a binding assay does not significantly exceed the Kd value. The lowest possible target concentration depends on the labeling affinity (Step A). If the target concentration in your binding assay cannot be adjusted to be below the Kd value, we recommend to determine an EC50 instead of a Kd value in your binding assay (Step C). If the affinity between labeling dye and your antibody of interest is too low (K_d > 5 nM) we recommend adjusting your assay buffer to improve the affinity or switching to a covalent labeling strategy for lysine (Cat. # NT-L111) or cysteine (Cat. # NT-L114) 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 buffer (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 buffer 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 antibody to each well/PCR-tube in reverse order (16-1) and mix by pipetting. The final concentration of labeled target in the assay is 20 nM. This concentration should be used for the calculation of the K_d value. If you are using Dianthus, spin the microwell plate and then seal the plate with sealing foil.
6. Measure your samples in Dianthus using auto-excitation or load the capillaries and measure the samples at 100 % LED/excitation power in Monolith. At the final dye concentration of 5 nM the expected fluorescence intensity at 100 % LED is around 400 counts on Monolith.

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 antibody prior to labeling has been overestimated, excess dye may be present. We recommend to re-check the concentration of your antibody or to increase the ratio between the antibody and the dye. We also recommend switching what buffer you are using as this can increase the delta response of the dye to antibody and antigen interaction.

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

   Yes, the prediluted labeling dye may be stored for about 8 weeks at -20 °C. We recommend freezing the solution in 100 µL aliquots.

3. *What is the impact of protein A on the labeling reaction?*

   Protein A and the labeling dye bind to the same region on human IgG antibodies. Protein A and the dye would be competing for the same binding site, and this would affect the labeling efficiency.

4. *What is the impact of BSA on the labeling reaction?*

   There has been an observation of unspecific binding of BSA to the human Fc labeling dye, concentrations of BSA above 100 nm interferes with the labeling of the target.

5. *Can I use labeling dye to label unpurified IgG Antibodies?*

   Yes, labeling unpurified IgG Antibodies with labeling dye is possible. It has been tested that the dye can successfully detect antibody-antigen binding in cell culture media and lysates. This should be tested prior to performing the binding assay (Step A, page 3). 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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2023-09-01_V001