HiLyte Fluor™ 555 Labeling Kit - NH_2 Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk14.pdf

General Information

HiLyte Fluor™ 555 Labeling Kit - NH_2 is primarily used for the preparation of HiLyte Fluor™ 555-labeled antibody for immunostaining and cellular proteins for tracing. NH_2-Reactive HiLyte Fluor™ 555, a component of this kit, has a succinimidyl ester group, and can easily make a covalent bond with an amino group of the target protein or other macromolecules without any activation process. Filtration Tube included in this kit is used for sample protein in removing small molecules such as Tris buffer and amine compounds that interfere with the assay or labeling reaction. The labeling process is very simple. Add the NH_2-Reactive HiLyte Fluor™ 555 to protein solution on a filter membrane, and incubate at 37°C for 10 min. Excess HiLyte Fluor™ 555 molecules can be removed by a Filtration Tube. The excitation and emission wavelengths of the HiLyte Fluor™ 555-labeled proteins are 555 nm and 570 nm, respectively. This kit contains necessary reagents for labeling, including the storage buffer for conjugates.

Kit Contents

- NH_2-Reactive HiLyte Fluor™ 555 ..... 3 tubes
- Reaction Buffer .....500 µl x 1
- WS Buffer .....4 ml x 1
- Filtration Tube .....3 tubes

Capacity

Three samples labeling
- Sample requirement: Molecular weight > 50,000; amount: 50-200 µg

Storage Condition

Store at 0-5°C. This kit is stable for 1 year at 0-5°C before opening.

Caution
After a NH_2-Reactive HiLyte Fluor™ 555 is taken out from the seal bag, keep the unused NH_2-Reactive HiLyte Fluor™ 555 (s) in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

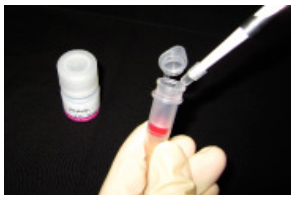
Required Equipment

- 10 µl, 200 µl adjustable pipettes
- Microcentrifuge
- Incubator (37°C)
- DMSO
- Microtubes

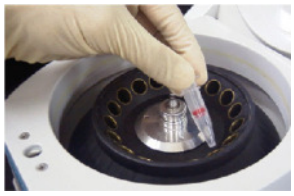
Precaution

- If the target protein solution contains other proteins with molecular weight larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for HiLyte Fluor™ 555 labeling, because it might interfere the labeling reaction.
- If the protein solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.
- The droplets which induced from the dry inhibitor of membrane, are occasionally found inside Filtration Tube while storing the kit at 0-5°C or after returning to room temperature. This phenomenon does not affect the performance.

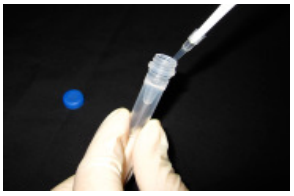
General Protocol for protein labeling



Step 1.
Add 100 µl WS Buffer and the sample solution containing 50-200 µg protein[a] to a Filtration Tube.

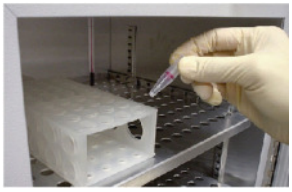


Step 2.
Mix the solution with pipetting several times, and centrifuge at 8,000 x *g* for 10 min.[b]

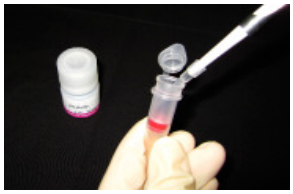


Step 3.
Add 10 µl DMSO to NH_2-Reactive HiLyte Fluor™ 555, and dissolve with pipetting.[c]

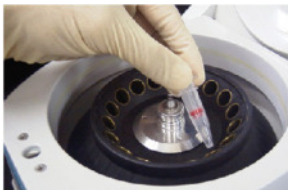
Step 4.
Add 100 µl Reaction Buffer to the Filtration tube, and then add 8 µl NH_2-Reactive HiLyte Fluor™ 555 solution[d] to the Filtration Tube and pipette to mix.



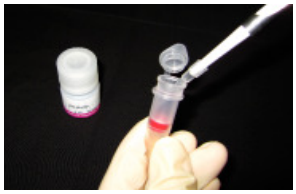
Step 5.
Incubate the tube at 37°C for 10 min.



Step 6.
Add 100 µl WS Buffer to the Filtration Tube, and centrifuge at 8,000 x *g* for 10 min.[b] Discard the filtrate.



Step 7.
Add 200 µl WS Buffer to the Filtration Tube, and centrifuge at 8,000 x *g* for 10 min.[b] Repeat this step one more time.



Step 8.
Add 200 µl WS Buffer, and pipette about 10 times to recover the conjugate.[e] Transfer the solution to a microtube (not included in this kit), and store at 0-5°C.

a) The volume of protein solution should be less than 100 µl. If the antibody concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total protein accumulation becomes 50 - 200 µg.
b) If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min.
c) NH_2-Reactive HiLyte Fluor™ 555 is on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve. NH_2-Reactive HiLyte Fluor™ 555 can be hydrolyzed by moisture in DMSO. Proceed to Step 4 immediately after the preparation of the NH_2-Reactive HiLyte Fluor™ 555 solution.
d) If the amount of protein is 200 µg, add entire NH_2-Reactive HiLyte Fluor™ 555 solution.
e) We recommend using WS Buffer to storage the conjugate. You can choose any kinds of buffers appropriate for your experiment. Though the membrane might be colored with unreacted dye, more than 90% of conjugate would be recovered after pipetting with WS Buffer.

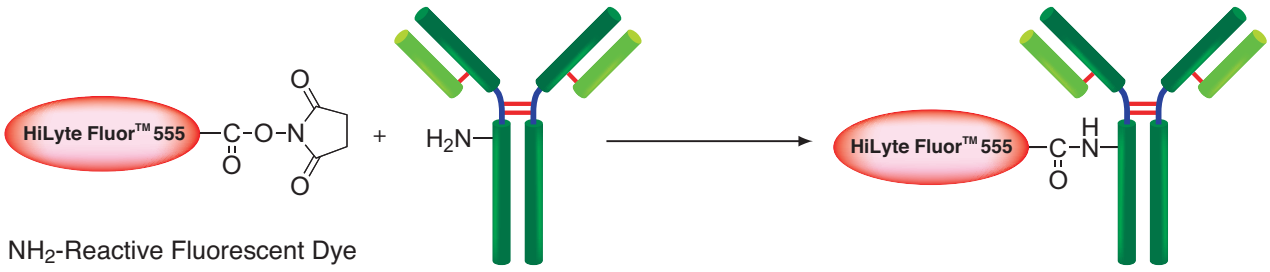
Determination of Dye / protein Ratio

Dilute the HiLyte Fluor™ 555-labeled protein solution with WS Buffer or other neutral buffer to a proper volume then measure the absorbance of the protein solution at 280 nm and 555 nm, if you need to know the HiLyte Fluor™ 555/protein ratio. Calculate the ratio using the following equation: When target protein is IgG, use 216,000 as the ε of IgG. Molar absorption coefficient of HiLyte Fluor™ 555 in WS Buffer is 150,000.

$$\text{Ratio (HiLyte Fluor}^{TM}\text{ 555 molecules per protein molecule)} = \frac{A_{555}/150{,}000}{(A_{280} - A_{555} \times 0.1)/(\varepsilon \text{ of protein})}$$

A_{555}: absorbance at 555 nm
A_{280}: absorbance at 280 nm
ε: molar absorption coefficient of protein at 280 nm

Labeling Reaction



NH_2-Reactive Fluorescent Dye

Q & A

- **Can I use this kit to label antibodies which is commercially available?**
  Yes. However, if the antibody solution contains other proteins such as serum albumin or gelatin, labeling reaction might be interefered by that protein. Purification of the antibody solution with affinity chromatography is necessary prior to use this kit.
  Contact us for the purification procedure, if you need.

- **How many HiLyte Fluor™ 555 molecules are introduced to protein?**
  The number of conjugated HiLyte Fluor™ 555 depends on the protein. In the case of rabbit IgG, 3 to 7 HiLyte Fluor™ 555 molecules conjugate to each protein molecule.

- **How long is the HiLyte Fluor™ 555-labeled protein stable?**
  Stability of conjugate depends on the protein itself. In the case of labeling for rabbit IgG, the labeled IgG is stable at 4°C for 2 months. For longer storage, add equal volume of glycerol to the sample solution and store at -20°C.

- **What is a minimum amount of protein that can be labeled using this kit?**
  We recommend using 50 µg as a minimum amount. Though 10 µg protein can be labeled using this kit, the background might be increased.

- **Can I use this kit to label oligonucleotides or oligopeptides?**
  No. Oligonucleotides and oligopeptides may be too small to retain on the membrane filter of Filtration Tube.

- **Is there any notice for treatment of living cells with the HiLyte Fluor™ 555-labeled protein?**
  We recommend using PBS including 2-10% FBS for preparation of cell suspension to maintain the best cell condition.

- **Does recovery buffer (WS Buffer) have harmful effect to living cells?**
  No. WS Buffer contains stabilizing agent (surfactant) that is controlled of its concentration without cytotoxicity. If you are concerned about the additive in WS Buffer, you can use your own buffer currently used instead of WS Buffer.

If you require assistance, please contact Dojindo customer service.

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