Ab-10 Rapid Biotin Labeling Kit

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk37.pdf

General Information

Ab-10 Rapid Biotin Labeling Kit enables rapid (in less than 30 min) and easy labeling of Biotin to 10 µg antibody. Reactive Biotin (a component of the kit) has succinimidyl ester group, that can easily make a covalent bond with an amino group of the target antibody without any activation process. This kit contains all the necessary reagents to prepare a Biotin-labeled antibody except for DMSO.

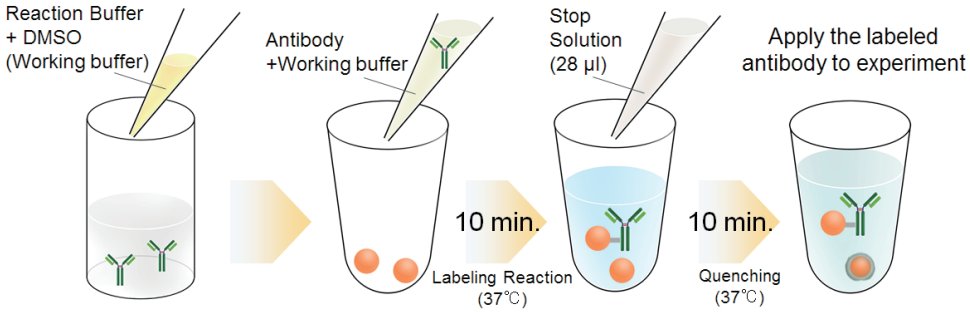

Fig. 1 Labeling procedure

Caution

After a Reactive Biotin is taken out from the seal bag, keep the unused Reactive Biotin in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

Kit Contents

- Reactive Biotin* x 3
- Reaction Buffer 100 µl x 1
- Stop Solution 100 µl x 1

*Though it is hard to see the reagent Reactive Biotin because of its small amount, it is attached on the bottom of the tube as a pellet form. Please collect the Reactive Biotin carefully by pipetting with buffer as described in Step 4 of the protocol.

Storage Condition

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

Required Equipment and Materials

- 20 µl, 200 µl adjustable pipettes
- Incubator (37 °C)
- DMSO (Dimethyl sulfoxide)
- Microtube (for sample and Working buffer preparation)
- PBS (Phosphate buffered saline)

Precaution

- Use 0.5-1 mg/ml of antibody solution for labeling. If the antibody concentration is more than 1 mg/ml, please dilute the antibody solution with PBS.
- If the sample solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.
- The microtubes in this kit contain solutions. Since there is a possibility that the droplets might attach to the inside walls or caps, please spin the tube to drop them down prior to open.
- In case an antibody solution includes a high concentration of constituents, such as BSA or glycerol, it may interfere with a labeling and cause a non-specific signal. We recommend removing the constituents prior to labeling procedure. Usable constituents (○) and non-usable constituents (×) are shown in Table 1, and compatible concentrations of constituents are shown in Table 2.

Table 1. Usable/non-usable constituents

Additives	
Buffering agents (PBS,HEPES)	○
Sodium chloride	○
Chelating agents (EDTA)	○
Sodium azide	○
Sugars (Glucose, Trehalose)	○
Primary amines and thiols	×

Table 2. Compatible concentrations of constituents

	Glycerol	BSA	Gelatin	Tris
Anti-Chloramphenicol Acetyl Transferase (CAT) antibody	< 20%	< 0.1%	< 0.1%	< 50 mmol/l
Anti-GAPDH antibody	< 50%	< 0.1%	< 0.1%	< 50 mmol/l
Anti-CD44 antibody	< 50%	< 0.5%	< 0.1%	< 50 mmol/l

Interference and non-specific signal may be dependent on types of antigen, host species of antibody or constituents.

Protocol

1. Add Reaction Buffer (up to 30 µl) to a microtube and mix it with an equal volume of DMSO to prepare Working buffer.
2. Add 0.5-1 mg/ml of the antibody solution to another microtube to be an amount of antibody of 10 µg.
3. Add Working buffer (step.1) to the antibody solution (step 2) and mix by pipetting.
   ※ The volume of Working buffer : one-fifth of the antibody solution (Table 3).
4. Add the solution (step 3) to Reactive Biotin and mix by pipetting.
5. Incubate at 37℃ for 10 minutes.
6. Add 28 µl of Stop Solution to the solution (step 5) and mix by pipetting.
7. Incubate at 37℃ for 10 minutes.
8. Apply the sample (step 7) for desired experiments or store at 0-5 ℃.
   ※ The labeled antibody is stable at 4℃ for 2 weeks.

Table 3. The volume of Working buffer

The concentration of antibody (mg/ml)	0.5	0.6	0.7	0.8	0.9	1.0
The volume of Working buffer (µl)	4.00	3.34	2.86	2.50	2.22	2.00

Supplimental Information

Mitochondria immunostaining

1. HeLa cells were seeded on a µ-slide 8 well (ibidi) and cultured overnight at 37 ℃ in a 5% CO_2 incubator.
2. The cells were washed with PBS three times, and 4% paraformaldehyde in PBS was added to the µ-slide.
3. The µ-slide was incubated at room temperature for 15 minutes.
4. The cells were washed with PBS three times, and 1% Triton-X in PBS was added to the µ-slide.
5. The µ-slide was incubated at room temperature for 30 minutes.
6. Once the cells were washed with PBS three times, a blocking solution prepared with PBS was added to the µ-slide.
7. The cells were then incubated at room temperature for 1 hour.
8. Biotin conjugated anti-mitochondria antibody was diluted 50 times with the blocking solution.
   ※ Anti-mitochondria antibody was purchased from Abcam (Product Code: ab3298) .
9. The supernatant was discarded and the solution (step 8) was added to the µ-slide.
10. The µ-slide was incubated at 0-5℃ overnight.
11. The supernatant was discarded and the cells were washed using PBS-T three times.
12. 0.2 µg/ml peroxidase conjugated streptavidin was added to the µ-slide.
13. The µ-slide was incubated at room temperature for 1 hour.
14. The supernatant was discarded and the cells were washed using PBS-T three times.
15. The cells were washed using Tris buffer (TB, 50 mmol/l, pH 7.5) three times.
16. The supernatant was discarded and DAB solution [0.2 mg/ml DAB (Dojindo Laboratories, Product Code:D006), 0.003% H_2O_2, 50 mmol/l Tris (pH 7.5)] was added to the µ-slide.
17. The µ-slide was incubated at room temperature for 10 minutes.
18. After the cells were washed using TB three times, TB was added to the µ-slide.
19. The cells were observed under a microscope.

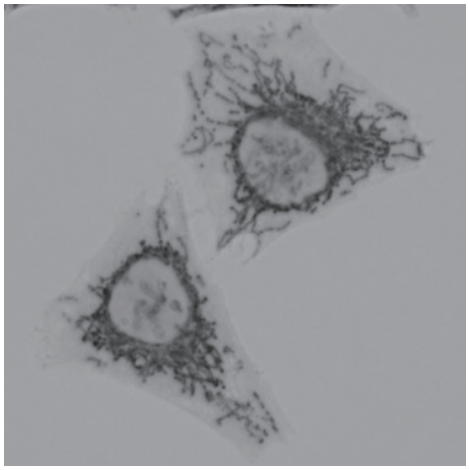

Fig. 2 Microscope image of DAB-stained mitochondria in HeLa cells

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