Ab-10 Rapid Peroxidase Labeling Kit *Technical Manual*

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk33.pdf

General Information

Ab-10 Rapid Peroxidase Labeling Kit is rapid (in less than 30 min) and easy preparation kit of peroxidase-labeled antibody (Ab) for 10 µg antibody. Reactive Peroxidase (a component of the kit) has succinimidyl ester groups, 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 peroxidase-labeled antibody.

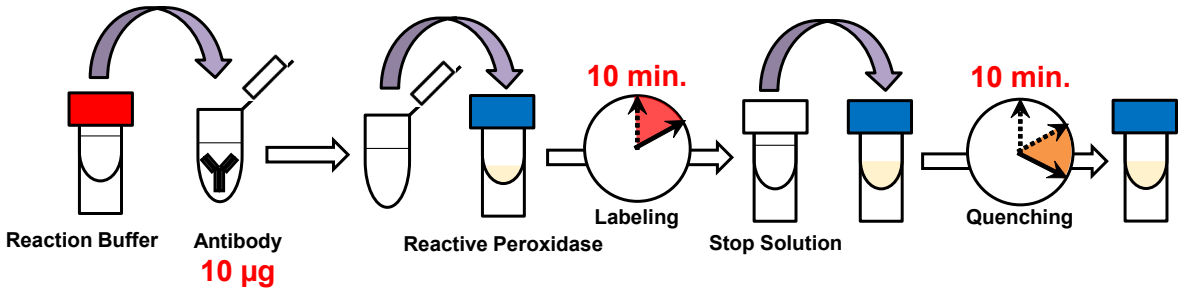

Fig. 1 Labeling procedure

Caution

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

Kit Contents

- Reactive Peroxidase x 3
- Reaction Buffer 100 µl x 1
- Stop Solution 100 µl x 1

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 adjustable pippet
- Incubator (37 °C)
- Microtube (for sample 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 down to drop them down prior to open.
- Some additives in an antibody solution may interfere with the labeling if the concentration is too high. The maximum compatible concentrations of such additives are indicated in Table 1.

Table 1. Compatible concentrations of the additives

Additives		Additives	
Buffering agents (PBS, HEPES)	○	Sodium azide	< 0.1%
Sodium chloride	○	BSA*	< 1%
Chelating agents (EDTA)	○	Gelatin	< 0.1%
Sugars (Glucose, Trehalose)	○	Tris	< 50 mmol/l
Glycerol	< 50%	Primary amines and thiols	×

∗ Containing BSA may result in non-specific signal depending on the antibodies used. Removing BSA prior to the peroxidase labeling is recommended in case high non-specific signal is observed.

Protocol

1. Add 0.5-1 mg/ml of the antibody solution to a microtube to be an amount of antibody of 10 µg.
2. Add Reaction Buffer to the antibody solution (step 1) and mix by pipetting.
   ※ The volume of Reaction Buffer: one-tenth of the antibody solution (Table 2).
3. Add the solution (step 2) to Reactive Peroxidase and mix by pipetting.
4. Incubate at 37°C for 10 minutes.
5. Add Stop Solution to the solution (step 4) and mix by pipetting.
   ※ The volume of Stop Solution: one-tenth of the antibody solution (Table 2).
6. Incubate at room temperature for 10 minutes.
7. Apply the sample (step 6) for desired experiments or store at 0-5 °C.
   ※ The labeled antibody is stable at 4°C for 2 weeks. For longer storage, add equal volume of glycerol to the sample solution and store at -20°C.

Table 2. The volume of Reaction Buffer and Stop Solution

The concentration of antibody (mg/ml)	0.5	0.6	0.7	0.8	0.9	1.0
The volume of Reaction Buffer (µl)	2.00	1.67	1.43	1.25	1.11	1.00
The volume of Stop Solution (µl)	2.00	1.67	1.43	1.25	1.11	1.00

Supplimental Information

Mitochondria immunostaining

1. HeLa cells were seeded on a µ-slide 8 well (ibidi) and cultured overnight at 37 °C in a 5% CO_2 incubator.
2. The cells were washed using PBS three times, and 4% paraformaldehyde in PBS was added to the µ-slide.
3. The cells were then incubated at room temperature for 1 hour.
4. The supernatant was discarded 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 0-5°C for 1 hour.
8. Peroxidase 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°C overnight.
11. The cells were washed using Tris buffer (TB, 50 mmol/l, pH 7.5) three times.
12. 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.
13. The µ-slide was incubated at room temperature for 10 minutes.
14. After the cells were washed using TB three times, TB was added to the µ-slide.
15. The cells were observed under a microscope.

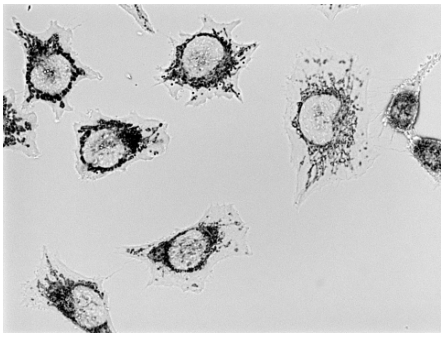

Fig. 2 Microscope image of mitochondria in HeLa cells

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