Ab-10 Rapid R-Phycoerythrin Labeling Kit

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk34.pdf

General Information

Ab-10 Rapid R-Phycoerythrin Labeling Kit is rapid (in less than 30 min) and easy preparation kit of R-Phycoerythrin-labeled antibody (Ab) for 10 µg antibody. Reactive R-Phycoerythrin (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 R-Phycoerythrin-labeled antibody.

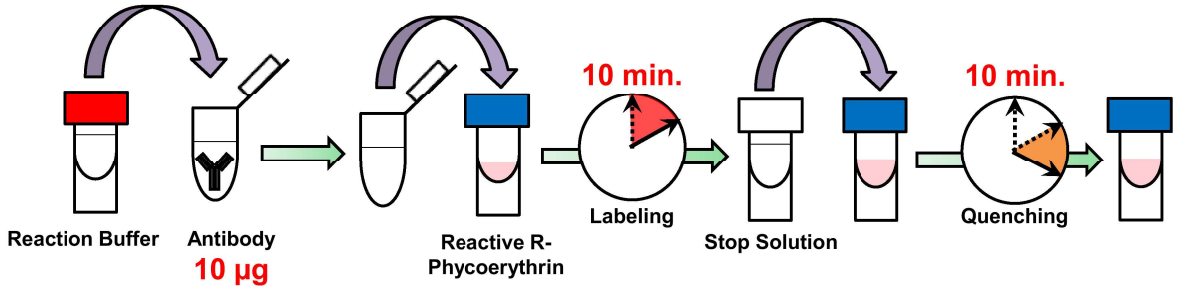

Fig. 1 Labeling procedure

Caution

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

Kit Contents

- Reactive R-Phycoerythrin	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 R-Phycoerythrin 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 R-Phycoerythrin 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

Fluorescent staining HL60 cells

1. HL60 cells were added to the number of 5 x 10^5 cell/tube in a microtube.
2. The supernatant was removed by centrifugation at 1,000 x *g* for 2 minutes.
3. Suspension buffer [50 µl, 1% FBS (fatal bovine serum), Hanks' HEPES balanced buffer] was added to the tube.
4. R-Phycoerythrin conjugated antibody (1 µg) was added to the tube and mixed by pipetting.
   ※ Anti-CD13 antibody was purchased from Becton Dickinson (Product Code:555393) . Mouse IgG (Isotype) was purcased from Jackson Immuno Research Laboratories (Product Code:015-000-003).
5. The tube was incubated on ice for 30 minutes.
6. The supernatant was removed by centrifugation at 1,000 x *g* for 2 minutes.
7. Suspension buffer (1 ml) was added to the tube and the cells were suspended by pipetting.
8. Stained cells were analysed by flow cytometry.

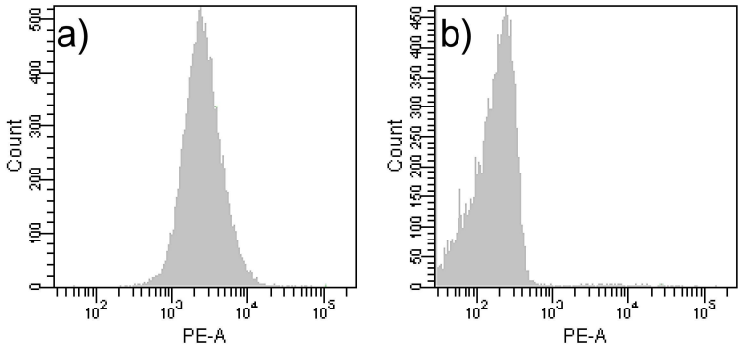

Fig. 2 Staining HL60 cells with R-Phycoerythrin labeled antibody
a) R-Phycoerythrin labeled-mouse anti-CD13 antibody
b) R-Phycoerythrin labeled-mouse antibody (Isotype)

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If you need more information, please contact Dojindo technical service.

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