Peroxidase Labeling Kit-NH_2

Introduction

Peroxidase Labeling Kit-NH_2 is for simple and rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA. NH_2-reactive peroxidase (a component of this kit) has an activated ester group, and can easily make a covalent bond with an amino group of the target molecule without any activation process. If the target is a small molecule, the conjugate can be purified with Filtration tube included in this kit. Filtration tube is also used for sample IgG in removing small molecules such as sodium azide, Tris buffer and amine compounds that interfere with the assay or labeling reaction. This kit contains all of the necessary reagents for peroxidase labeling, including the storage buffer for conjugates.

Cat.Number:	#BT377	BT3771 1kit 3 rxn / 100µg	BT3772 1kit 1 rxn/1mg	BT3773 1kit 1/2 x 10/5mg
Description	**HRP Labeling kit-NH_2**			
	Kit contains: - NH2-reactive peroxidase	100 µg x 3	1mg x 1	10mg
	- Washing buffer	4 ml x 1	10ml x 1	50ml
	- Reaction buffer	200 µl x 1	1.2ml x 1	12ml
	- Storage buffer	4 ml x 1	10ml x 1	50ml
	- Filtration tube	3 tubes	1 tube 15ml centrif.tube	2 tubes
Capacity:	Protein (Molecular weight > 50000, IgG: 50-200 µg) Small molecule (Molecular weight < 5000)			
Storage condition	Store at 0-5 °C. This kit is stable for 6 months at 0-5 °C with protection from moisture.			
Equipment and material non provided	- 10 µl, 200 µl and 1 ml pipettes - Incubator (37°C) - Microcentrifuge			

Labeling Procedure for IgG.

(*for kit BT3771/1 labeling of 50-200µg IgG*)

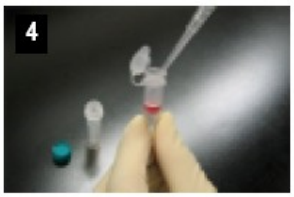
1. Add the sample solution containing 50-200 µg IgG[a)] and 100 µl Washing buffer to Filtration tube.
2. Mix the solution with pipetting several times, and centrifuge at 8 000 g for 10 min[b)].
3. Add 100 µl Washing buffer, and centrifuge again at 8 000 g for 10 min[b)].
4. Add 10 µl Reaction buffer to a lyophilized NH_2-reactive peroxidase and dissolve it with pipetting.
5. Transfer the solution containing NH_2-reactive peroxidase onto the membrane of Filtration tube where IgG is concentrated.
6. Rinse the entire surface of the membrane with the solution by pipetting, and incubate the tube at 37°C for 2 hr.
7. Add 100 µl Washing buffer to the tube, and centrifuge at 8 000 g for 10 min[b)].
8. Add 200 µl Storage buffer[c)], and pipette several times to recover the conjugate. Transfer the solution to a 500 µl tube (not included in this kit), and store the solution at 0-5°C [d)].

a) The recommended amount of IgG is 100 µg. The volume of sample solution should be less than 100 µl. If the antibody concentration is lower than 0.5 mg/ml, repeat step 1 and 2 until the total IgG accumulation becomes 50-200 µg. If the sample solution contains other proteins such as BSA, purify the antibody prior to using this kit.
b) If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min.
c) The concentration of the conjugate is 0.5-1.3 mg/ml. Dilute the peroxidase-labeled IgG to prepare a solution with an appropriate concentration prior to using it for enzyme immunoassay, immunoblotting or immunostaining. One to three molecules of peroxidase should be introduced onto one IgG molecule. Unconjugated peroxidase should not interfere with normal immunoassays. If purification is necessary, use a gel permeation column or an affinity column for IgG.
d) Generally, the peroxidase-labeled IgG in Storage buffer is stable for at least 2 months at 0-5 °C. For longer storage, add glycerol (final concentration: 50%), aliquot, and store at -20 °C. However, it is important to note that the stability will depend on the sample itself.

Add 100 µl Washing buffer and the sample solution containing 50-200 µg IgG to a Filtration tube.[a)]

Centrifuge at 8,000-10,000 g for 10 min. Add 100 µl Washing buffer and centrifuge once more.[b)]

Add 10 µl Reaction buffer to NH_2-reactive peroxidase and dissolve with pipetting.



Transfer the solution containing NH_2-reactive peroxidase onto the membrane of the filtration tube where IgG is concentrated.

Rinse the entire surface of the membrane with the solution by pipetting and incubate the tube at 37 °C for 2 hrs.

Add 100 µl Washing buffer to the tube. If the volume of the filtrate is 300 µl or more, discard the filtrate prior to go to Step 7.

Centrifuge at 8,000-10,000 g for 10 min.[b)]

Add 200 µl Storage buffer and pipette 10 to 15 times to recover the conjugate.[c)] Transfer the solution to a 0.5 ml tube and store the solution at 0-5 °C.[d)]

Labeling Procedure for Small Molecule with Amino Group

(*for kit BT3771/1 labeling*)

1. Prepare 50 µl of 2 mM amine compound solution[a)] with Reaction buffer.
2. Add this solution to a tube of NH_2-reactive peroxidase.
3. Pipette several times to dissolve NH_2-reactive peroxidase completely, and incubate at 37°C for 1 hr.
4. Add 100 µl Washing buffer to the reaction solution, and transfer the solution to Filtration tube.
5. Centrifuge the tube at 8 000 g for 10 min[b)].
6. Discard the filtrate, and then add 200 µl Washing buffer to the tube.
7. Centrifuge the tube again at 8 000 g for 10 min[b)].
8. Repeat Steps 5 and 6.
9. Add 200 µl Storage buffer, and pipette several times to dissolve the conjugate[c)].
10. Transfer the solution to a 500 µl tube (not included in this kit), and store the solution at 0-5°C [d)].

Prepare 50 µl of 1 mM amine compound solution with Reaction buffer,[a)] and add the solution to a tube of NH_2-reactive peroxidase. Pipette several times to mix, and incubate at 37 °C for 1 hr.

Add 100 µl Washing buffer to the reaction solution and transfer the entire solution to a filtration tube.

Centrifuge at 8,000-10,000 g for 10 min.[b)] Discard the filtrate. Add 200 µl Washing buffer to the tube and centrifuge at 8,000-10,000 g for 10 min.[b)] Add 200 µl Washing buffer and centrifuge again.

Add 200 µl Storage buffer, and pipette 10 to 15 times to recover the conjugate.[c)] Transfer the solution to a 0.5 ml tube and store the solution at 0-5 °C.[d)]

a) If the amine compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mM solution, and mix 5 µl of this solution with 45 µl Reaction buffer.
b) If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min.
c) The concentration of the conjugate is about 400-500 µg/ml (10-12.5 µM). One to two target molecules should be conjugated with one peroxidase molecule.
d) The peroxidase-labeled small molecule should be stable for at least 6 months at 0-5 °C.

Frequently asked questions

- **Can I use this kit for other proteins or peptides?**
  Yes, if the molecular weight is higher than 50 000 or lower than 5 000, and it has a reactive primary or secondary amino group. If the molecular weight is higher than 50 000, follow the labeling protocol for IgG, and use 0.5-1 nmol of sample protein. If it is lower than 5 000, follow the labeling protocol for small molecules. If the molecular weight is lower than 50 000 but higher than 5 000, contact our customer service at Interbiotech@interchim.com for more information.

- **Can I use this kit to label an oligonucleotide?**
  Yes, if the molecular weight is less than 5,000, and it has a reactive primary or secondary amino group. Follow the labeling protocol for small molecules.

- **What is the minimum amount of IgG that can be labeled with this kit?**
  The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. Though 10 µg IgG can still be labeled using this kit, the background will be higher.

- **How many peroxidase molecules per IgG are introduced?**
  Average number of peroxidase molecule per IgG is 1 to 3.

- **Does unconjugated NH_2-reactive peroxidase still have an activated ester after the labeling reaction to IgG?**
  No. It is completely hydrolyzed during the reaction. That is one of the reasons why blocking or purification steps are not necessary.

- **Does NH_2-reactive peroxidase form an oligomer during the labeling reaction?**
  No. Since all amino groups of NH2-reactive peroxidase are blocked, no oligomerization is possible.

- **Do I have to use Storage buffer included with the kit?**

No, you do not have to use Storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment.

Other information

For R&D use in vitro only.
Also available in kit format of 3x100µg labeling (#BT3772) and for 5/10mg labeling (#BT3773) .

Related product:

HRP labeling kit-SH (#BT7691)

For more information, please ask interbiotech@interchim.com