

HRP Antibody Labeling Kit (100 µg/Reaction)

【Catalog】 ALK-A005

【Size】 1 Reaction; 3 Reactions

Please read this manual carefully before performing the experiment.

For research use only, not for use in diagnostic or therapeutic procedures.

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Catalog

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【Introduction】

The HRP Antibody Labeling Kit enables efficient and rapid conjugation of antibodies to horseradish peroxidase (HRP) via primary amine groups (such as lysine) on the antibody surface. The entire operation process is simple, and the conjugation product can be obtained in just 3.5 hours without any purification steps and without loss of antibodies. The resulting product can be directly used in ELISA experiments.

Note: The conjugates generated by the HRP Antibody Labeling Kit are not compatible with ELISA assays using streptavidin-coated plates.

【Catalog Number and Specification】

Table 1. Catalog number and Specification

Catalog Number	Size	Specification
ALK-A005	1 Reaction	1 reaction, 100 µg antibody labeling per reaction
ALK-A005	3 Reactions	3 reactions, 100 µg antibody labeling per reaction

【Precautions】

1. For research use only, not for use in diagnostic procedures.
2. Please use the kit within the shelf life.
3. Components of different kits and different batches of kits should not be mixed.
4. This kit is only used for the labeling of purified antibodies. Antibodies in ascites, serum, hybridoma or tissue culture may affect the labeling result.
5. This kit is designed only for antibody labeling.

【Materials Provided】

Table 2. Materials provided

Catalog Number	Size	Component ID	Components	Component Size (1 kit)	Format	Storage
ALK-A005	1 Reaction	ALK-A005-C01	Activated HRP	1 vial	Powder	-20°C
		ALK-A005-C02	Crosslinking Reagent	1 vial	Powder	
		ALK-A005-C03	Modifier Reagent	1 vial	Liquid	
		ALK-A005-C04	Quencher Reagent	1 vial	Liquid	
ALK-A005	3 Reactions	ALK-A005-C01	Activated HRP	1 vial × 3	Powder	-20°C
		ALK-A005-C02	Crosslinking Reagent	1 vial × 3	Powder	
		ALK-A005-C03	Modifier Reagent	1 vial	Liquid	
		ALK-A005-C04	Quencher Reagent	1 vial	Liquid	

【Storage】

Store the kit at -20°C upon receipt. Please use it prior to the expiration date indicated on the packaging.

【Unsupplied Reagents or Equipment】

1. Single micropipettes and pipette tips: need to meet 10 µL, 100 µL, 1000 µL injection requirements.
2. Thermostatic shaking incubator.
3. Vortex mixer.
4. Tubes: 1.5 mL, 2 mL.

【Conjugation Protocol】

1. Preparation of antibody

Prepare the antibody at 1 mg/mL in 1× PBS (pH 7.2-7.4).

Note 1: For optimal conjugation performance, the recommended antibody concentration should be used. Higher or lower concentrations may affect the conjugation efficiency.

Note 2: Keep the antibody solution away from amine-containing substances such as Tris, glycine, BSA or gelatin.

Table 3. Buffer composition requirements

Buffer composition	Yes or No
Buffer without amine groups	Yes
pH 6.5-8.5	Yes
EDTA	Yes
Sugars	≤10%
Tris	No
Glycerol	<50%
Sodium azide	No
Proclin	No
BSA or gelatin	No
β -mercaptoethanol or DTT	No
Note: The optimal labeling buffer is 1× PBS (pH 7.2-7.4).	

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2. Antibody Conjugation

2.1 Before the experiment, take out component C01, C02 and C03, and equilibrate them to room temperature. Centrifuge the C03 vial at $1000 \times g$ for 1 minute to collect the liquid at the bottom of the tube.

***Note:** It is normal if crystals form in the C03 component, allow it to remain at room temperature (or in a room temperature water bath) until the crystals completely disappear, then vortex to mix.*

2.2 Add 100 μL of antibody solution (1 mg/mL, from Step 1) to the C02 vial. Gently pipet or vortex until the lyophilized powder is fully dissolved and well mixed.

2.3 Add 10 μL of component C03 to the C02 vial. Mix by gently pipetting or vortexing.

2.4 Transfer the entire solution from the C02 vial (containing the antibody, C02, and C03) to the C01 vial. Mix by gentle pipetting or vortexing until the lyophilized powder is completely dissolved.

***Note:** Perform steps 2.2 to 2.4 consecutively without prolonged intervals.*

2.5 Close the lid and place the vial in a shaking incubator. Incubate at 23°C for 3 hours at 150 rpm, protected from light.

***Note:** If a shaking incubator is not available, mix by pipetting up and down every 1.5 hours.*

3. Reaction Quenching

3.1 Equilibrate component C04 to room temperature before use. Centrifuge the C04 vial at $1000 \times g$ for 1 minute to collect the liquid at the bottom of the tube.

3.2 Add 10 μL of component C04 to the C01 vial (from Step 2.5). Mix by gently pipetting or vortexing.

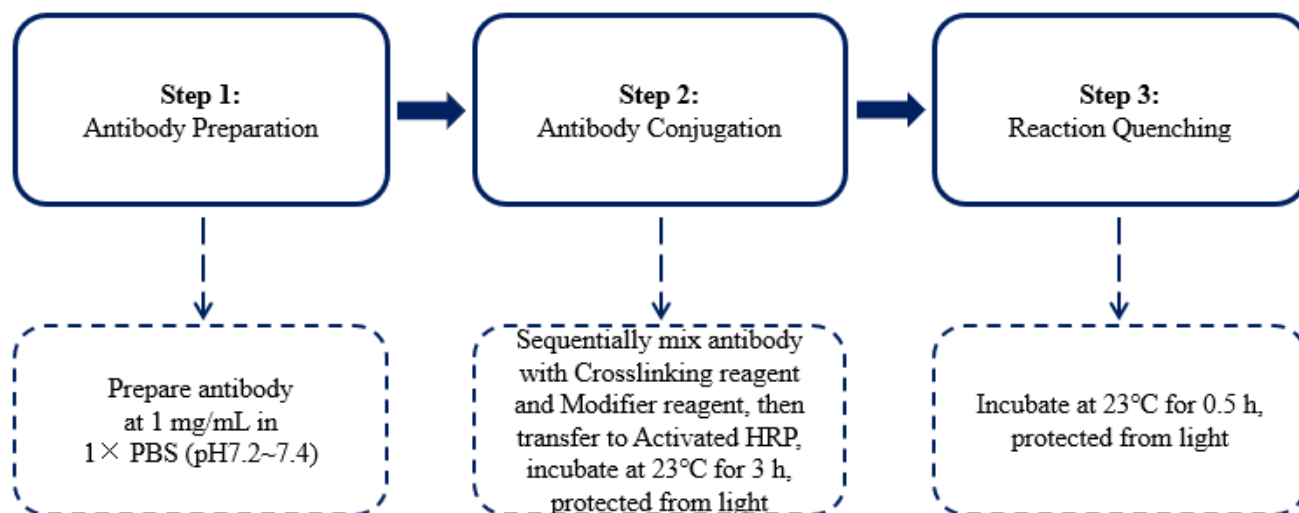
3.3 Close the lid and place it in a shaking incubator. Incubate at 23°C for 0.5 hour at 150 rpm, protected from light, and the conjugated antibody will be obtained.

***Note:** If a shaking incubator is not available, mix by pipetting up and down every 15 minutes.*

【Storage of Conjugate】

It is recommended to add 50% glycerol to the conjugated antibody, aliquot, and store at -80°C, protected from light.

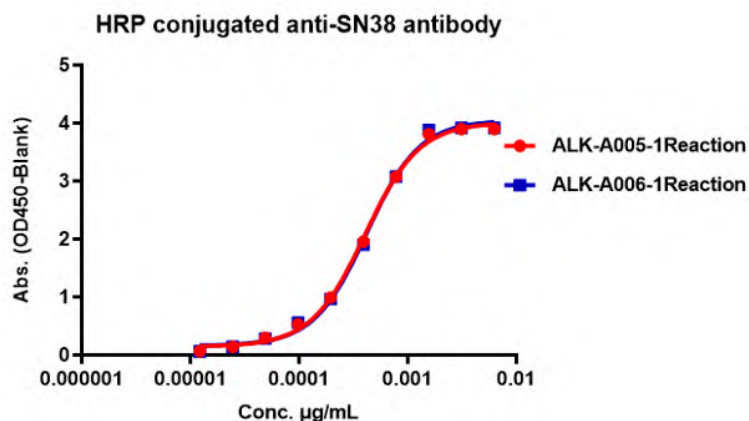
【Quick Guild】



【Typical Data】

The following figure shows the data of products using ALK-A005-1Reaction / ALK-A006-1Reaction.

Conc. ($\mu\text{g/mL}$)	ALK-A005- 1Reaction	ALK-A006- 1Reaction
0.00625	4.000	4.000
0.003125	4.000	4.000
0.0015625	3.914	3.962
0.00078125	3.175	3.156
0.000390625	2.059	1.983
0.000195313	1.090	1.045
9.76563E-05	0.628	0.638
4.88281E-05	0.395	0.362
2.44141E-05	0.242	0.221
1.2207E-05	0.165	0.143
Blank	0.101	0.080
EC50 ($\mu\text{g/mL}$)	0.0004048	0.0004177
RSD (EC50)	2%	



For each experiment, the specific MFI value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only.