# FlyLink™ HRP Labeling Kit

Cat #: D-AKE100

Size: 100 μg x 3

Storage: Stored at 4°C for 6 months, protected from light

## Assay Principle

Horseradish peroxidase (HRP) is a 44,173.9-dalton glycoprotein with 6 lysine residues which can be conjugated to a labeled molecule. It produces a colored, fluorometric, or luminescent derivative of the labeled molecule when incubated with a proper substrate, allowing it to be detected and quantified. HRP is often used in conjugates to determine the presence of a molecular target. HRP is also commonly used in techniques such as ELISA and Immunohistochemistry due to its monomeric nature and the ease with which it produces colored products.

FlyLink™ HRP Labeling Kit is designed for preparing HRP conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The HRP provided in our kit has been pre-activated, and can be directly used for conjugation. The coupling product can be stored stably for 1 month at 4°C.

**Application example:** The coupled direct-labeled primary antibody does not require a secondary antibody in the immunoassay, thus eliminating tedious incubation and washing steps, saving time.

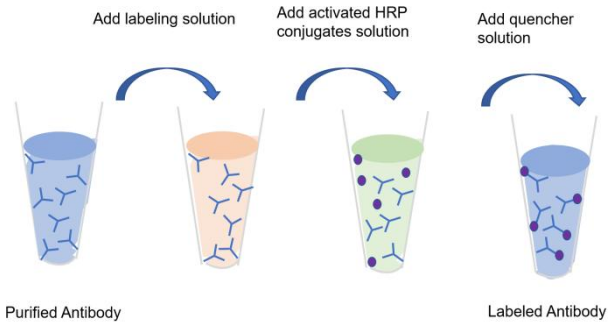


Fig.1: Schematic diagram of FlyLink™ HRP Labeling Kit

**Applicable samples:** labeling biomolecules, especially proteins, peptides, and other ligands that contain a free amino group

## Materials Supplied and Storage Conditions

| Kit components | Size（100 μg） | Storage conditions |
|---|---|---|
| Activated HRP solution | 22.5 μL | 4°C, protected from light |
| HRP labeling solution | 30 μL | 4°C |
| HRP quencher powder | 3 | 4°C, protected from light |
| Booklet | 1 | RT |

## Materials Required but Not Supplied

·Prepared samples to be labelled, Deionized water

·Precision pipettes, Disposable pipette tips, Ultrafiltration tubes or dialysis bags (if needed)

## Sample Preparation

**List of components (or storage buffer) requirements of the sample to be labeled:**

| pH | 6.5-8.0 |
|---|---|
| Amine free buffer | MES, PBS, HEPES |
| Chelating agents (e. g. EDTA) | ✓ |
| Glycerol | < 50% |
| Sodium Azide | ✗ |
| BSA | < 0.1% |
| Glycine | ✗ |
| Gelatin | < 0.1% |
| Proclin | ✗ |
| Borate buffer | ✓ |
| Components containing amino | ✗ |

**Note: that components that containing amino are not allowed in the buffer, which will affect the coupling effects. Sodium**

**Azide will destroy HRP, also Proclin should be removed from the coupling buffer. A small number of BSA and gelatin will not affect the results, and it is recommended to use PBS as the required buffer. If the sample contains substances that may interfere with the labeling, it is suggested to replace the buffer with PBS. The specific method is as below. Add the sample to the ultrafiltration tube and add 200-150 µL PBS. Centrifuge at 12,000 g, 4°C, 10 min, and discard the filtrate. Then PBS was added again, and centrifuge at 12,000 g, 4°C, 10 min. After centrifugation, the inner core of the ultrafiltration tube is taken out, placed it in a clean outer tube, and centrifuge at 4,000 g, 4°C, 2 min to collect the sample.**

## Assay Procedure

1. Setting up your conjugation reactions

The amounts of molecules used for labeling ideally should correspond to molar ratios between 1:1 and 1:4 molecules to HRP. We suggest you start conjugation with an approximately 1:3 ratio. For optimum molar ration, you could conduct preliminary experiments to confirm it. For example, the amount of Antibody for different ratios, from 1:1 to 1:4 are as follows.

| Size | Amount of HRP | Amount of antibody (Ab: HRP) | | | Optimal Labeling system |
|---|---|---|---|---|---|
| | | 1:1 | 1:3 | 1:4 | |
| 3×100 µg/Kit | 3×75 µg | 3×300 µg | 3×100 µg | 3×75 µg | 3×100 µL |
| 1 mg/Kit | 750 µg | 3 mg | 1 mg | 750 µg | 1 mL |

Taking account of the molecular weights (160,000 versus 40,000), this means for that for 1 mg HRP you need to add between 1-4 mg of antibody.

To guarantee coupling effects, the original concentration should more than 1 mg/mL. For other sizes, the amount will be magnified in equal proportion referring to the above table.

Take the 3×100 µg /Kit for example, the recommended ratio of antibody to be labeled and HRP enzyme is 1:3. For other sizes, the amount of the components will be adjusted in equal proportion referring to the procedures. If you want to adjust the molar ratios, keeping the amount of HRP constant, and you could change the amounts of molecules and buffer dosages according to the above table.

(1) Add 30 µL of HRP labeling solution to the antibody solution to be labeled. Mix gently using a pipette.

(2) Remove the screw cap from the tube of Activated HRP solution and pipette 22.5 µL to the solution of Step (1), add

deionized water to the volume of 300 µL. Mix gently and leave it standing in the dark at room temperature (20-25°C) for 2 h.

(3) Add 1 mL deionized water to HRP quencher powder to generate 100× concentrated quencher solution. The Quencher solution could be placed at room temperature, but should be used within 48 h. Quencher working solution should be diluted 100 times before use.

**Note: Please prepare the quencher solution with lid open to prevent tube burst, due to gas accumulation in the tube and expand instantly; The amount of deionized water be constant (1 mL) upon different sizes of kits.**

(4) Add 45 µL quencher working solution to the solution of Step (2), mix gently and standing for 2 h at 4°C avoiding the light. The conjugate can be used after this. No separation steps are necessary.

2. Storage of conjugates

Your HRP conjugate can be stable at 4°C for more than one month. For longer storage, the conjugate can be stored at -20°C with cryoprotectant such as 50% glycerol. According to your following experiments, the conjugates can be purified by using ultra-filtration column. And the best conditions for any special conjugates must be determined by experimentation.

## FAQ

**Q1: The reaction volume exceeds the optimal coupling system because of low concentration of antibodies?**

**A1:** If the concentration is still less than 1 mg/mL after the concentrate, adjust the reaction volume properly, but the final concentration should be more than 0.5 mg/mL. The HRP labeling solution should be 10% of the final volume, and don't add deionized water in the second procedure. But the Activated HRP solution and Quencher solution should remain unchanged.

**Q2: The molar ratios of molecules with HRP can only between 1:1 and 1:4?**

**A2:** For different molecules, the suitable molar ratios are disparate. For antibodies, we suggest it should not exceed 1:4. For other molecules, you could try unlike rations according to your own requirements.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.