

HRP Conjugation Kit

v. 240101

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|--------------------------|---|-------------------|--------------------|
| Catalog number | C08001-10 / C08001-100 / C08001-1000 | | |
| Package | 10 µg / 100 µg / 1 mg | | |
| Description | <p>Horseradish peroxidase (HRP) is a 44 kDa glycoprotein that can be modified to crosslink with antibodies or other proteins, without compromising the enzymatic activity. HRP conjugates can be widely used in ELISA, western blotting and IHC due to its enzymatic characteristics. Croyez HRP Conjugation Kit is designed for HRP conjugation of a small quantity (10 µg-1 mg) of antibody or protein. It provides a rapid and easy process with high efficiency to conjugate HRP to antibody or protein. The kit provides all the necessary components and high-activity HRP for conjugation.</p> | | |
| Component | C08001-10 | C08001-100 | C08001-1000 |
| HRP | 1 vial | 1 vial | 1 vial |
| 10X Modifier | 1 vial | 1 vial | 1 vial |
| Reduction reagent | 1 vial | 1 vial | 1 vial |
| 10X Quencher | 1 vial | 1 vial | 1 vial |
| Storage | Store at -20°C. | | |
| Note | <p>Common non-buffering salts (e.g., sodium chloride) do not affect conjugation efficiency. Avoid buffer components containing primary amine (e.g., amino acid or ethanolamine) and thiols (e.g., 2-Mercaptoethanol or DTT).</p> <p>Components that have no effect or little effect on labeling reaction:</p> <ul style="list-style-type: none"> • up to 20 mM Tris • up to 10% glycerol • up to 0.02% sodium azide | | |

Antibody concentrations of 1-4 mg/mL generally give optimal results.

Recommended amount and volume of antibody for optimal results.

| Kit size | Antibody amount | Reaction volume |
|-----------------|------------------------|------------------------|
| 10 µg | 10-20 µg | 4-20 µL |
| 100 µg | 100-200 µg | 40-200 µL |
| 1 mg | 1-2 mg | 400-2000 µL |

HRP conjugation protocol

1. Dissolve antibody in PBS or other buffer that do not contain amine, Tris, NaN₃ or glycerol. Add **10X Modifier** to antibody (e.g. 1 μL of 10X HRP modifier for 9 μL of antibody).
2. Spin down the vial of **HRP** before use.
3. Make sure all buffers are well dissolved before use. If not, please vortex the vial to make salts dissolved.
4. Remove cap of the vial of **HRP** and pipette antibody into the vial. Mix gently by pipetting several times.
5. Cover the cap on the vial and incubate in the dark at room temperature for 3 hours.
6. After incubating, add 1/20 volume of **Reduction reagent** (e.g. 1 μL of reduction reagent for 20 μL of antibody-HRP mixture) and mix gently by pipetting. Incubate at room temperature for 30 minutes.
7. Add **10X Quencher** (e.g. 1 μL of 10X Quencher for 9 μL of antibody-HRP mixture) and mix gently by pipetting. The conjugates can be used after 30 minutes.

Manual

* For protein conjugation, the amount of protein can be calculated by formula below:

Quantities of protein = quantities of kit (e.g. 10 μg) x (M.W. of target protein)/(150 (M.W. of IgG))

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