

DESCRIPTION:

Activated HRP labeling kit enables the direct conjugation of antibodies, proteins and peptides, or any other biomolecule with an available amine group, to Horseradish Peroxidase (HRP) with high sensitivity, high yield and low background. The technology is fully scalable from 10 µg to 1 g or more and stringently QC tested for consistent high quality and excellent batch-to-batch reproducibility.

COMPONENTS:

Each kit contains the components necessary for at least five labeling reactions:

- Activated HRP Lyophilized Powder
- Reaction Buffer (150 mM NaHCO₃, pH 9.5 with preservative)
- Reduction Buffer (5 M Sodium Cyanoborohydride solution)
- Quenching Buffer (1 M Glycine with preservative)

PROTOCOL:

The following protocol is optimized for immunoglobulin labeling and gives a 4 fold HRP to IgG molar excess labeling ratio. The molar ratio of HRP to protein can be optimized for specific proteins or applications.

1. IgG Preparation: Prepare 1 mg IgG in Reaction Buffer for a final concentration of 1 mg/mL and a final volume of 1 mL. See Note 1.
2. Activated HRP Preparation: Add 50 µL Reaction Buffer to one unit of lyophilized activated HRP powder.
3. Conjugation Reaction: Add the IgG solution to the HRP and gently vortex to mix well. Incubate the reaction for 2-3 hours at room temperature with gentle agitation. See Note 2.
4. Quenching Reaction: Add 2 µL of Sodium Cyanoborohydride to the reaction in a fume hood then add 10 µL of Quenching Buffer. Incubate at room temperature for 15 minutes.
5. Clean up: Dialyze or desalt the conjugate into the desired buffer (e.g. PBS).

6. Storage: Store conjugate at 4°C for up to 4 weeks. For long term storage, add either bovine serum albumin at 5 mg/mL or an equal volume of glycerol. Prepare single use aliquots and store at -20°C.

Notes:

1. The starting concentration of the IgG solution must be at least 1.2 mg/mL before diluting it with Reaction Buffer to achieve optimal pH for labeling. A more dilute IgG solution can be buffer exchanged into Reaction Buffer and concentrated to achieve a final concentration of 1 mg/mL.
2. If less than 1 mg of IgG is to be labeled, use proportionally less activated HRP in the reaction.

STORAGE: 2 - 8°C