

Maleimide Activated HRP

CATALOG #: M1322-1 1 mg M1322-5 5 mg

ACTIVATION METHOD: Sulfo-SMCC

MOL. WEIGHT: ~ 40 kDa

pH: 7.2 ± 0.2

FORM: Lyophilized

BINDING CAPACITY: ~2-4 mg of peptide (average MW of 1000 – 3000) per 2 mg HRP

RECONSTITUTION: Reconstitute in distilled water at the concentration of 2-5 mg/ml with gentle stirring.

STORAGE CONDITIONS: Lyophilized maleimide-activated HRP should be stored at -20°C and is stable for 1 year. Reconstitute

right before use.

Note: Use the reconstituted protein immediately. Do not store it.

DESCRIPTION: Maleimide Activated Horseradish Peroxidase (HRP) is commonly used to facilitate the conjugation of

HRP to proteins, peptides and ligands that contain sulfhydryl (-SH) groups for use as an enzyme-linked detection reagent. This product consists of HRP that has been modified with the hetero bi-functional cross linker Sulfo-SMCC and it generally contains several maleimide groups per HRP molecule while maintaining most of the HRP activity. After conjugation, the activated HRP will form a covalent interaction with the peptides or ligands containing sulfhydryl groups and can be used as a HRP probe

for detection in different assays such as ELISA and western blot etc.

APPLICATIONS: ELISA, Western blot

PROTOCOL:

PROCEDURE FOR PEPTIDE CONJUGATION:

1. Dissolve the sulfhydryl-containing hapten in 0.2-0.5 ml of phosphate buffer pH 7.2.

Note: For haptens with limited solubility in phosphate buffer add DMSO (do not exceed DMSO 15% of total volume). Alkaline pH values (above 8.5) may hydrolyze the maleimide group or generate side reactions with amines. Haptens must contain cystenine or a sulfhydryl group in the reduced state in order to react efficiently with maleimide group.

 Thaw the Maleimide Activated HRP at room temperature and dissolve it in distilled H₂O right before use to yield a concentration of 2-5 mg/ml.

Note: Do not vortex vigorously or heat the activated HRP.

- 3. Immediately mix the peptide (2.5 mg) with activated HRP (2.5 mg) and incubate at room temperature for 2 hrs under occasional mixing.
- 4. Peptide-conjugated HRP can be purified by gel filtration or dialysis to remove unconjugated peptide.

Note: If the peptide-conjugated HRP is to be stored for > 2 weeks, it is recommended to store at -20° C. The peptide-conjugated HRP can be stored at -20° C for more than 6 months. Avoid repeated freeze-thaw.

 The coupling efficiency of conjugation can be determined by assaying the content of free sulfhydryl groups in the unreacted peptide using Sulfo-SMCC reagent.

RELATED PRODUCTS:

- Maleimide Activated KLH (M1317)
- Maleimide Activated OVA (M1318)
- Maleimide Activated BSA (M1316)

FOR RESEARCH USE ONLY! Not to be used in humans.