

Protein-maleimide Conjugates for Thiol-maleimide Crosslinking

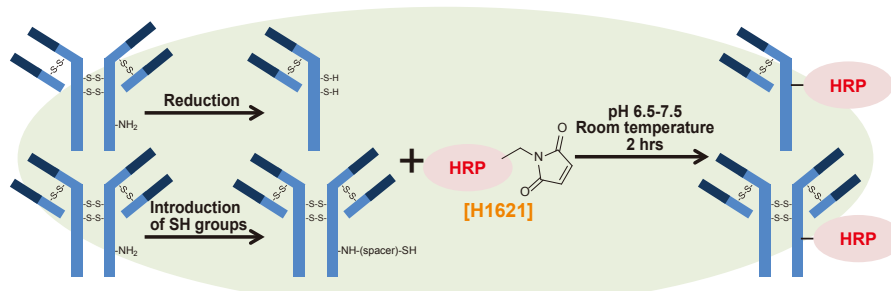
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)	1set [H1621]
Bovine Serum Albumin Maleimide Conjugate (1mg×3)	1set [B5944]
Streptavidin Maleimide Conjugate (0.5mg×1)	1vial [T3531]

Advantages

- Each product containing a thiol-reactive maleimide group can be used for the conjugation to proteins and peptides containing free thiols.
- Each protein conjugate is packaged for single use purposes and thus does not require weighing prior to use.

Application : HRP-labelling of an antibody with **H1621**

In case of antibodies without free thiol (SH, sulfhydryl) groups, disulfide moieties in proteins can be reduced by a reductant such as DTT **[D3647]** or 2-MEA **[A0296]** to reveal free thiols. Furthermore, thiol group can be introduced to primary amines by adding SATA **[S0431]**, SATP **[S0859]** or 2-Iminothiolane.

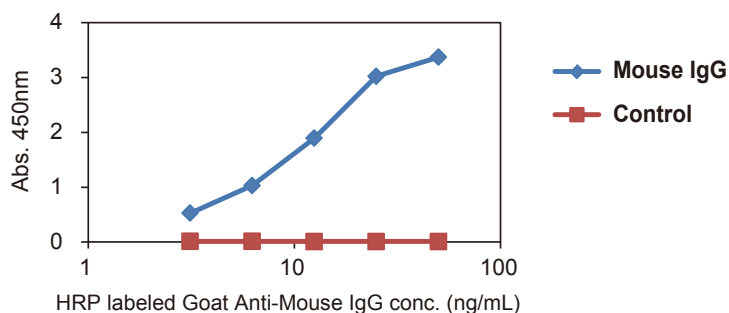


Example protocol for antibody conjugation starts from a reduction of native disulfide bonds in the Goat Anti-Mouse IgG, followed by labeling with the HRP using **H1621**. For more information, see the product detail page of **H1621** on TCI website.

Protocol

- 1) Add DTT to a final concentration equal to 3 mole equivalents per mole equivalent of antibody present.
- 2) Incubate for 90 minutes at 37 °C.
- 3) Purify the reduced IgG by gel filtration or ultrafiltration, dialysis.
- 4) Add equal amount of **H1621** (by weight) to a purified antibody and incubate for 2 hours at room temperature (25 °C).

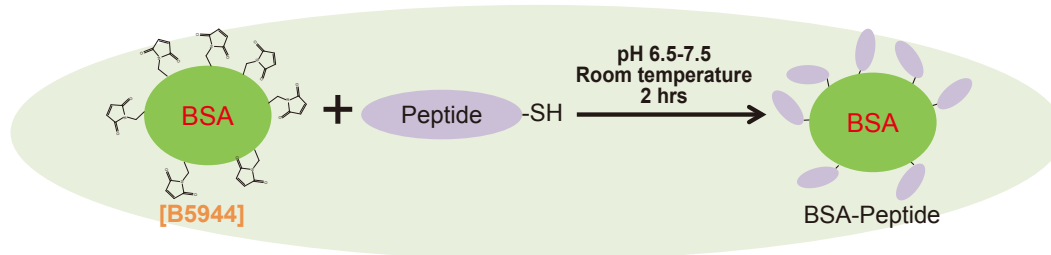
Activity of HRP labeled antibody



Goat Anti-Mouse IgG labeled with the HRP using **H1621** was tested by ELISA for detection of a Mouse IgG coated on a plate. Mouse IgG could be detected sufficiently even if the labeled antibody was diluted to 5 ng/mL or more.

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Application : Preparation of BSA-Peptide using **B5944**



BSA is conjugated to haptens and typically used as an antigen carrier for anti-hapten antibody.

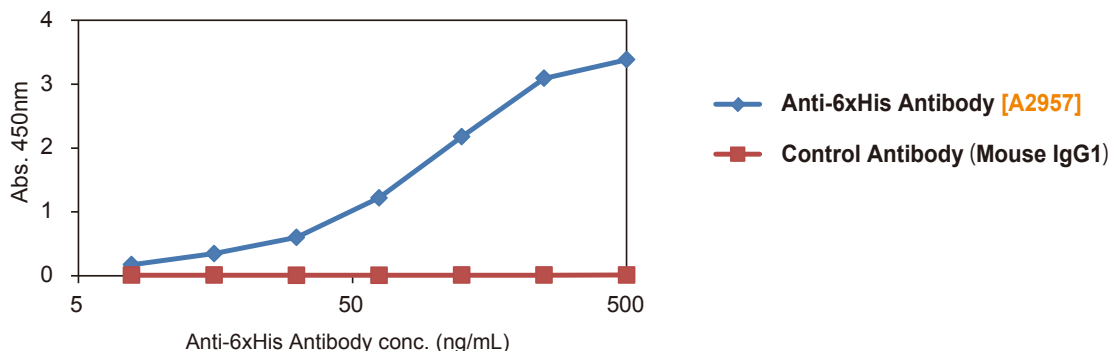
Here we show how to conjugate 6xHis-Cys peptide to **B5944**.

For more information, see the product detail page of **B5944** on TCI website.

Protocol

- 1) Dissolve the 6xHis-Cys peptide in 0.1 M sodium phosphate, 0.15 M NaCl, 0.1 M EDTA at pH 7.2.
- 2) Reconstitute the **B5944** with 100 μ L of water.
- 3) Add 1mg of 6xHis-Cys peptide to 1 mg of **B5944** and Incubate for 2 hours at room temperature (25 $^{\circ}$ C).

ELISA using the prepared BSA-6His as an antigen



Anti-6xHis Antibody **[A2957]** was analysed by ELISA using a 0.1 μ g / well of BSA-6His coated plate.

Goat Anti-Mouse IgG HRP Conjugate **[G0407]** was used as the secondary antibody.

Related Products

Reducing agents for protein disulfide

DTT (= DL-Dithiothreitol)	1g / 5g [D3647]
2-MEA (= 2-Aminoethanethiol Hydrochloride)	25g / 100g / 500g [A0296]
2-Mercaptoethanol	5g / 25g [M1948]
Tris(2-carboxyethyl)phosphine Hydrochloride	1g / 5g / 25g [T1656]

Reagents for introduction of thiol group

SATA (= N-Succinimidyl S-Acetylthioglycolate)	1g / 5g [S0431]
SATP (= N-Succinimidyl 3-(Acetylthio)propionate)	100mg [S0859]

For further information please refer to our website at www.TCIchemicals.com.

maleimide conjugate



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