

Peroxidase (HRP) Labeling Reagents

Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)

1set [H1621]

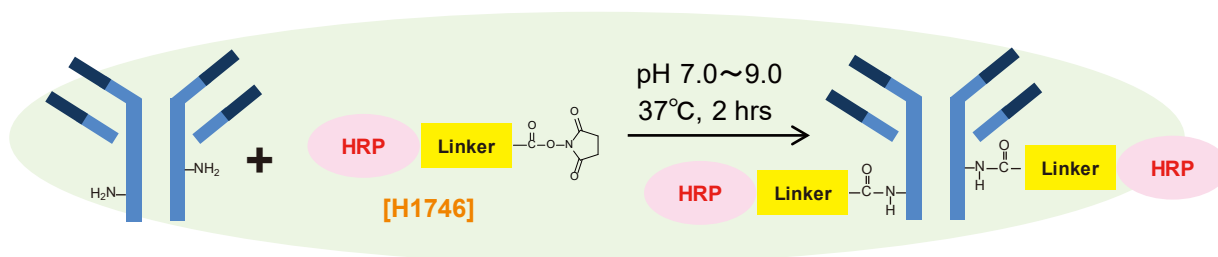
Horseradish Peroxidase NHS Ester Conjugate (0.2mg×3)

1set [H1746]

Advantages

- **H1746** contains an *N*-hydroxysuccinimidyl ester (NHS) moiety. It can be used to readily label proteins and peptides that have an amino group (-NH₂).
- **H1621** 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-labeling of an antibody with H1746



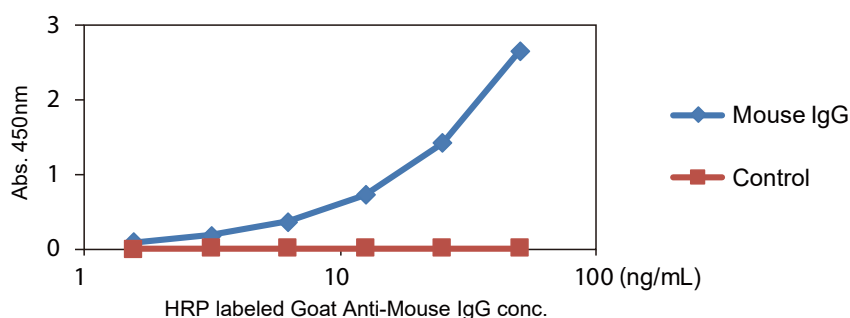
Here is an example of HRP labeling of an antibody (Goat Anti-Mouse IgG) conjugated with **H1746**. For more information, see the product detail page of **H1746** on TCI website.

Protocol

- 1) Dissolve the target antibody at 10 mg/mL in 0.1 M Sodium Bicarbonate buffer (pH 8.5)*.
- 2) Add the antibody solution into **H1746** vial, and mix well.
- 3) Incubate for 2 hours at 37 °C.
- 4) To quench the reaction, add 200 μL of 100mM Tris-HCl buffer pH 7.5.
- 5) Incubate for 1 hour at 37 °C.

*Tris buffer and other amine containing buffers also interfere with the labeling reaction. We recommend using the amine free buffer (e.g PBS, Phosphate buffer, Borate buffer, Bicarbonate buffer) pH range 7-9.

Activity of HRP labeled antibody

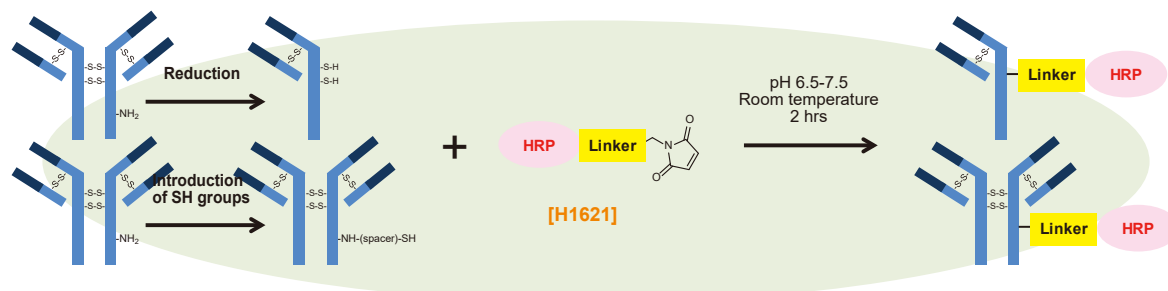


Goat Anti-Mouse IgG labeled with the HRP using **H1746** was tested by ELISA for detection of a Mouse IgG coated on a plate. Mouse IgG could be detected sufficiently even if the labeled antibody was diluted to 15 ng/mL or more.

Peroxidase (HRP) Labeling Reagents

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.

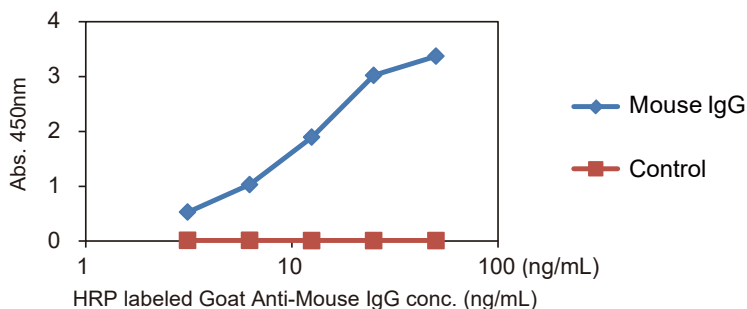


Example protocol for antibody conjugation starts from a reduction of native disulfide bonds in the Goat Anti-Mouse IgG, followed by labeling with the HRP using H1621. For more information, see the product detail page of H1621 on TCI website.

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.

Related Products

Bovine Serum Albumin Maleimide Conjugate (1mg×3)
Streptavidin Maleimide Conjugate (0.5mg×1)

1set [B5944]
 1vial [T3531]

Reducing agents for protein disulfide

DTT (= DL-Dithiothreitol)
2-MEA (= 2-Aminoethanethiol Hydrochloride)
2-Mercaptoethanol
Tris(2-carboxyethyl)phosphine Hydrochloride

1g / 5g [D3647]
 25g / 100g / 500g [A0296]
 5g / 25g [M1948]
 1g / 5g / 25g [T1656]

Reagents for introduction of thiol group

SATA (= N-Succinimidyl S-Acetylthioglycolate)
SATP (= N-Succinimidyl 3-(Acetylthio)propionate)

1g / 5g [S0431]
 100mg [S0859]

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



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