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ProductInformation

ANTI-HSV
Developed in Rabbit, Affinity Isolated Antibody

Product Number **H 6030**

Product Description

Anti-HSV is developed in rabbits using a synthetic peptide (K-QPELAPEDPED) conjugated to KLH via the N-terminal Lysine. The peptide corresponds to amino acids 290-300 of Glycoprotein D precursor which is an envelope component of herpes simplex virus.[1] The antibody is affinity purified using the immunizing peptide immobilized on resin.

Anti-HSV antibody reacts specifically with HSV tagged fusion proteins, applying immunoblotting and immunoprecipitation techniques. Reaction of the antibody in immunoblotting is inhibited by the HSV immunizing peptide (Product No. H 4640).

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that provide 'affinity handles' (tags) designed to enable the selective identification and purification of the protein of interest.[2-7] The addition of a tag to a given gene creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the biodistribution of the tagged product.

Reagent

The product is provided as a solution of approx. 0.8 mg/ml affinity isolated antibody in 0.01 M phosphate buffered saline pH 7.4 containing 1 % bovine serum albumin and 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

At least 2.5 µg/ml of the antibody detects by immunoblotting HSV tagged fusion protein in bacterial lyastes.

At least 1 µg of antibody can immunoprecipitate an HSV tagged fusion protein from bacterial lysates.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

Procedures

A. Procedure for Immunoblotting
Perform the entire procedure at room temperature.

1. Separate HSV tagged proteins from sample lysate using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5 – 20 µg total lysate per lane. The amount of cell extract or bacterial lysate to be loaded per lane depends on the level of expression of the tagged protein and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5 % to 10 % non-fat dry milk in PBS (Product No. D 8537) for 1 hour.

4. Wash the membrane at least three times for 5 minutes each in PBS containing 0.05 % Tween 20 (Product No. P 3563).
5. Incubate the membrane with anti-HSV antibody as the primary antibody in PBS containing 0.05 % Tween 20 with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
7. Incubate the membrane with anti-rabbit IgG peroxidase conjugate (Product No. A 0545) or Alkaline Phosphatase cunjugate (Product No. A 9919) as the secondary antibody or with an HRP-protein A conjugate (Product No. P 8651), at the recommended concentration in PBS containing 0.05 % Tween 20. Incubate for 60 minutes. Adjust the concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
9. Treat the membrane with a peroxidase or an alkaline phosphatase substrate.

B. Procedure for Immunoprecipitation

Note: The amount of cell extract or bacterial lysate to be used for immunoprecipition depends on the level of expression of tagged protein and the specific application.

1. Centrifuge 25 µl Protein A - agarose beads (Product No. P 3476) for 1 min 12,000 x *g*, and then wash twice with 1 ml lysis buffer or PBS at 4 °C.
2. Add Anti-HSV antibody diluted in PBS (Product No. D 8537), to its recommeded concentration and incubate on a rotator for 1 hour.
3. Centrifuge 1 min at 12,000 x *g*, and then wash twice with 1 ml lysis buffer or PBS at 4 °C.
4. Add 0.1 ml to 1 ml cell extract or lysate (clarified by pre-spinning) containing HSV tagged protein to the beads and incubate on a rotator for 2 hours to overnight at 4 °C.
5. Spin down beads; remove supernatant.
6. Wash beads five times with 1 ml PBS each by vortex and short spin.
7. Resuspend the pellet in 25 µl 2X SDS-PAGE sample buffer (Product No. S 3401). Boil sample for 5 min and spin down. The supernatant is then ready to be loaded on an SDS-PAGE gel.

References

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