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Product Information

Anti-VP16
produced in rabbit, IgG fraction of antiserum

Catalog Number **V4388**

Product Description

Anti-VP16 is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 474-487 of the herpes simplex virus VP16 protein, conjugated to KLH via an N-terminal added cysteine residue. Whole antiserum is fractionated and further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-VP16 recognizes VP16 fusion proteins by immunoblotting and immunoprecipitation. Staining of VP16 fusion proteins by immunoblotting is specifically inhibited by the immunizing peptide.

VP16 from herpes simple virus (HSV) is a strong transcriptional activator of immediate early viral genes.[1] Due to its lack of DNA binding activity, VP16 is recruited to DNA via interaction with DNA binding proteins.[2] Ptashne, et al., showed that VP16 can function as a potent transcriptional activator in mammalian cells when fused to the DNA binding domain of the yeast transcriptional activator GAL4.[3] These properties were taken into consideration in the development of a mammalian version of the Yeast Two-Hybrid System.[4-6]

The Yeast Two-Hybrid System is a powerful method for characterizing and screening for protein-protein interactions. In the original version of the system, GAL4 DNA binding domain is fused to protein X, and GAL4 activation domain is fused to protein Y. Neither hybrid is capable of activating transcription individually. Upon interaction between proteins X and Y, the DNA binding and activator domains are brought in close proximity. As a result, the transcriptional activity of GAL4 is reconstituted at the promoter, and monitored with a reporter gene.[4]

Additional two-hybrid systems were developed using different DNA binding and activation domains as well as different reporter genes.[5-9] The VP16 activator is used in yeast and mammalian systems. In yeast, its DNA interaction partner is typically LexA.[10] In mammalian systems, interaction between two proteins is detected through recruitment of VP16 and GAL4 DNA binding domain to GAL4 operators present in the reporter gene.[7,9-11]

Antibodies specific for VP16 are useful tools for following and identifying such interactions.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or 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

Immunoblotting: a minimum working dilution of 1:1,000 is determined using mammalian cell extracts expressing VP16 fusion proteins.

Immunoprecipitation: 10 µg of the antibody can immunoprecipitate VP16 tagged fusion proteins from mammalian cell extracts expressing VP16 fusion proteins.

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

Procedures

Procedure for Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate VP16 tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in phosphate buffered saline (PBS), Catalog Number D8537, for at least 60 minutes.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Anti-VP16 antibody as the primary antibody for two hours, using an optimized concentration in PBS containing 0.05 % TWEEN.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane for one hour with Anti-Rabbit IgG-Peroxidase, Catalog Number A0545, as the secondary antibody, at the recommended concentration, in PBS containing 0.05% TWEEN 20. If necessary, adjust the antibody concentration to maximize detection sensitivity and 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 substrate according to the protocol provided with the substrate.

Procedure for Immunoprecipitation

As an alternative to the following protocol, Protein G Immunoprecipitation Kit, Catalo Number IP50, can be used to perform immunoprecipitation.

1. Centrifuge 40 µL of a 1:1 suspension of Protein G-Sepharose beads, Catalog Number P3296, for 1 min at 12,000 x *g*, and then wash twice with 1 ml RIPA buffer (50 mM Tris base, 0.25% w/v deoxycholate, 1% NP40, 150 mM NaCl, 1mM EDTA, pH 7.4) at 4 °C.
2. Add Anti-VP16 antibody diluted in PBS, and incubate on a rotator for 1 hour.
3. Centrifuge 1 min at 12,000 x *g*, wash twice with 1 ml RIPA at 4 °C.
4. Add 0.1-1.0 ml of cell extract containing VP16 tagged protein to the beads, and incubate from 2 hours to overnight at 4 °C on a rotator.
   Note: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.
5. Spin down beads; remove supernatant.
6. Wash beads five times with 1 ml PBS each by gentle vortex followed by a short centrifugation.
7. Resuspend pellet in 25 µL 2x SDS-PAGE sample buffer. Boil sample for 5 min and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

References

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