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ProductInformation

Anti-B42
Developed in Rabbit, Affinity Isolated Antibody

Product Number **B9808**

Product Description

Anti-B42 is developed in rabbits using as immunogen, a synthetic peptide corresponding to amino acids 58-72 of the *E. coli* B42 activation sequence, conjugated to KLH via an N-terminal added lysine residue. The antibody is affinity purified on the immunizing peptide immobilized on agarose.

Anti-B42 antibody reacts specifically with B42-fusion proteins. Specific staining is inhibited by the B42 immunizing peptide.

The 79 amino acid peptide B42 is one of several short peptides encoded by *E. coli* genomic DNA fragments, which have been shown to act as transcriptional activators in yeast when fused to the DNA binding domain of yeast GAL4 (GAL4 1-147).[1,2] B42 is capable of activating transcription when fused to LexA as well.[2] LexA is a 202 amino acid *E. coli* protein with a DNA binding activity that plays a central role in the regulation of the SOS response to DNA damage.[3,4] The ability of B42 to activate transcription in yeast, the LexA DNA binding activity, the modular nature of transcription factors and additional findings, altogether led to the development of the “LexA Yeast Two-Hybrid System”.[2,5]

The Yeast Two-Hybrid System is a unique system for studying and screening for protein-protein interactions. It is based on transcription factors in which the DNA binding and activator domains are functionally independent.[6,7] The DNA binding domain can be then fused to protein X (bait), whereas the activation domain can be fused to protein Y (prey), neither hybrid being capable of activating transcription independently. In cases where X and Y proteins interact with each other, DNA binding and activator domains are brought in close proximity. As a result, the transcriptional activity of bait and prey is reconstituted, and the interaction is monitored by an appropriate reporter gene.[6,7] There are several systems based on this principle, one of them being the LexA Two-Hybrid System. In this system, the DNA binding domain in the fusion protein is provided by LexA (protein X, bait), whereas the activation domain is the bacterial B42 peptide, which works in yeast, as indicated above.[1,2,5] The interaction between prey and bait is monitored using LacZ and LEU2 as reporter genes, under the control of LexA operators. Several interacting proteins have been isolated using this system.[5,8-12] Antibodies specific for B42 are a useful tool for following and identifying such protein interactions.

Reagent

The product is provided as a solution of affinity purified antibody in 0.01 M phosphate buffered saline pH 7.4 containing 1% bovine serum albumin and 15 mM sodium azide as a preservative.

Antibody Concentration: approx: 1.0 mg/ml

Precautions and Disclaimer

Due to the sodium azide content a material safety 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 2-8 °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

By immunoblotting, 0.5-1.0 µg/ml of the antibody detects 25 ng of purified recombinant B42 fusion protein, using a chemiluminescence substrate.

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

Procedure for Immunoblotting

Note: The entire procedure is performed at room temperature.

1. Separate B42 fusion proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol.
   Note: the amount of extract depends on the level of expression of the fusion protein and the specific application.
2. Transfer proteins from the gel to a nitrocellulose membrane.
4. Block the membrane using a solution of PBS containing 5% non-fat dry milk (Product No. P 4739) for at least 60 minutes.
5. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Product No. P 3563).
6. Incubate the membrane with Anti-B42 antibody as the primary antibody in PBS containing 0.05% TWEEN 20, with agitation for 120 minutes.
7. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
8. Incubate the membrane with anti-rabbit IgG, peroxidase conjugate (e.g. Product No. A 0545) as the secondary antibody at the recommended concentration in PBS, containing 0.05% TWEEN 20. Incubate with agitation for 60 minutes. Adjust the product concentration to maximize detection sensitivity and to minimize background.
9. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
10. Treat the membrane with a peroxidase substrate such as Sigma's Chemiluminescent Peroxidase Substrate (CPS-1) or use the ProteoQwest Chemiluminescent Western Blotting Kit (PQ0201) for added convenience.

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