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Product Information

Anti-GAL4 DNA-BD
produced in rabbit, affinity isolated antibody

Catalog Number **G3042**

Product Description

Anti-GAL4 DNA-BD is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 39-52 of *Saccharomyces cerevisiae* GAL4 protein, conjugated to maleimide activated Keyhole Limpet Hemocyanin (KLH) via a C terminal added cysteine residue. The peptide is derived from the DNA binding domain of GAL4. The antibody is affinity purified on the immunizing peptide immobilized on agarose.

Anti-GAL4 DNA-BD recognizes GAL4 DNA binding domain fusion proteins by immunoblotting. Specific staining is inhibited by the GAL4 DNA-BD immunizing peptide.

GAL4 protein is an 881 amino acid transcription factor involved in the induction of genes that regulate galactose metabolism in *Saccharomyces cerevisiae*. Similar to many transcriptional activators, its N-terminal DNA binding (147 amino acids) and C-terminal activator domains are functionally independent. Based on these properties, a unique system for studying and screening protein-protein interactions was developed. In the primary version of the "Yeast Two Hybrid System", the GAL 4 DNA-BD is fused to protein X (bait) and GAL4 activation domain is fused to protein Y (prey). Neither hybrid is capable of activating transcription independently. However, if X and Y proteins interact, the DNA binding and activator domains are brought in close proximity, and as a result, the transcriptional activity of GAL4 is reconstituted.[1-4] Transcription of an appropriate reporter gene, e.g., LacZ or HIS3, containing upstream GAL4 binding sites, is used to monitor the interaction between the two proteins.[1,7]

The yeast two hybrid system is widely used for several other applications, such as the characterization of domains that are necessary and sufficient for the interaction of two known proteins by deletion and mutational analysis.[5] Derivatives of this system, e.g., a one-hybrid system, can be used to characterize and screen for transcriptional activators when the cDNA is fused to the respective DNA-binding domain that is specific for the given yeast promoter-reporter gene construct.[6] Antibodies specific for the GAL4 DNA-BD are a useful tool for following and identifying such protein interactions.

Reagent

Solution in 0.01 M phosphate buffered saline pH 7.4 containing 1% bovine serum albumin and 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 at –20 °C. 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 of 1-2 µg/mL of the antibody detects GAL4 (DBD 1-147) fusion protein .

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

Procedure

Immunoblotting

Note: The whole procedure is performed at room temperature.

1. Separate GAL4 DNA-BD fusion proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 µg total lysate protein per lane.
   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.
3. Block the membrane using a solution of 3% non-fat dry milk in phosphate buffered saline (DPBS, Catalog Number D8537) for 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Anti-GAL4 DNA-BD 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-Alkaline Phosphatase, Catalog Number A9919, 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.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with an alkaline phosphatase substrate.

References

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