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ProductInformation

Anti-GAL4-AD (Activation domain)
Developed in Rabbit, Affinity Isolated Antibody

Product Number: **G 9293**

TECHNICAL BULLETIN

Product Description

Anti-GAL4-AD (activation domain) is developed in rabbits using a synthetic peptide corresponding to amino acids 867-881 of *Saccharomyces cerevisiae* GAL4 protein, conjugated to maleimide activated KLH via a cysteine residue added at the C-terminal. The peptide is derived from the activation domain domain of GAL4. The antibody is affinity purified on the immunizing peptide immobilized on agarose.

Anti-GAL4-AD antibody recognizes GAL4 DNA activation domain fusion proteins by immunoblotting. Specific staining is inhibited by the GAL4-AD 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 hybrids are 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-AD are a useful tool for following and identifying such protein interactions.

Reagents

The product is provided as a solution of affinity isolated 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.

Procedure

The whole procedure should be performed at room temperature.

1. Separate GAL4-AD fusion protein proteins from sample lysates using a standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 µg total lysate protein/lane.
   Transfer proteins from the gel to a nitrocellulose membrane.
   The amount of extract loaded 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 Dulbecco phosphate buffered saline containing 5% non-fat dry milk (DPBS, Product No. D 8537; non-fat dried milk, Product No. M 7409) for 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with an optimized concentration of Anti-GAL4-AD, diluted in DPBS containing 0.05% Tween 20 and 5% non-fat dry milk 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) 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. It is important to include a control in which no anti-GAL4-AD is added.
8. Wash the membrane three times for 5minutes each in PBS containing 0.05% Tween 20.
9. Treat the membrane with an alkaline phosphatase substrate.

Product Profile
At least 0.5 µg/ml of the antibody detects, by immunoblotting, GAL4-AD fusion protein in *Saccharomyces cerevisiae* extract.

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

References

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