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ProductInformation

Anti-LexA
Developed in Rabbit, Affinity Isolated Antibody

Product Number **L 0415**

Product Description

Anti-LexA is developed in rabbits using as immunogen a synthetic peptide corresponding to amino acids 164-180 of *E. coli* LexA protein with N-terminal added lysine, conjugated to KLH. The antibody is affinity purified using immunizing peptide immobilized on agarose.

Anti-LexA reacts with LexA (approx. 27 kDa), by immunoblotting. A cross-reactive band of approximately 62 kDa may be observed in some *S. cerevisiae* strains. Staining is inhibited by the LexA immunizing peptide.

The SOS response is a set of inducible reactions important for the survival of *E. coli* bacteria after treatment with various DNA-damaging agents.[1] LexA is a 202 amino acid protein which plays a central role in the regulation of the SOS response.[1,2] Under normal growth conditions, LexA functions as a repressor of transcription. In the event that the genome integrity is challenged by DNA damage, the SOS response is triggered. In essence, RecA stimulates the cleavage of LexA, which in turn leads to derepression of the SOS pathway.[3,4]

Information gathered from the research of LexA biological activity led to the development of the "LexA Yeast Two Hybrid System".[5-7] The Yeast Two Hybrid System is a unique way to study and screen protein-protein interactions. Its primary version was based on the yeast GAL4 transcriptional activator.[5] Similar to many other transcription factors, the DNA binding and activator domains are functionally independent. The GAL4 binding domain can be then fused to "protein X" (bait), whereas the GAL4 activation domain can be fused to "protein Y" (prey), such that neither hybrid alone is capable of activating transcription. If proteins X and Y interact, the GAL4 DNA binding and activator domains are brought in close proximity. As a result, the transcriptional activity of GAL4 is reconstituted, and the interaction is monitored with an appropriate reporter gene.[5,6] A similar rationale is applied to LexA Two Hybrid screening. In this system, the DNA binding domain in the fusion protein (with protein X, bait) is provided by LexA, whereas the activation domain is usually B42, a short *E.coli* activator sequence previously reported to work as a transcriptional activator in yeast.[7,8] The interaction between prey and bait is monitored using LacZ and LEU2 as reporter genes, under the control of LexA operators.[9-11] Antibodies specific for LexA are useful tools for following and identifying such protein interactions.

Reagent

The product is provided as a solution of affinity isolated antibody in 0.01M phosphate buffered saline (PBS) 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 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 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, at least 1.0 µg/ml of the antibody detects LexA expressed from a GAL1 promoter in *S. cerevisiae* extracts after induction with galactose.

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 LexA 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 PBS containing 5% nonfat dry milk (Product No. P 4739) for at least 60 minutes.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with Anti-LexA antibody as the primary antibody in PBS containing 0.05% Tween 20, with agitation for 120 minutes.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween 20.
7. 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.
8. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween 20.
9. Treat the membrane with a peroxidase substrate.

References

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