

Product Information

ANTI-c-Myc AGAROSE CONJUGATE

Developed in Rabbit, Affinity Isolated Antibody

Product Number **A 7470**

Product Description

Anti-c-Myc Agarose is prepared with an affinity purified anti-c-Myc antibody, developed in rabbit, coupled to cyanogen bromide-activated agarose. The purified antibody is immobilized at 1.0 to 1.5 mg antibody per ml agarose. Anti-c-Myc was developed in rabbit using a peptide corresponding to amino acid residues 408-425 of human c-Myc as the immunogen.

Anti-c-Myc recognizes an epitope located on c-Myc tagged fusion proteins. The antibody reacts specifically with N- and C- terminal c-Myc-tagged fusion proteins and may be used for the immunoprecipitation or immunoaffinity purification.

The human *c-myc* proto-oncogene is the human cellular homolog of the avian *v-myc* gene found in several leukemogenic retroviruses.^{1,2,3} Increased expression of the cellular oncogene *c-myc* has been described in a variety of human tumors, occurring by several mechanisms, including gene amplification and chromosomal translocation.³

An epitope located within amino acid residues 410-419 (EQKLISEEDL) of human *c-myc* has been widely used as a tag in many expression vectors, enabling the expression of proteins as c-Myc tagged fusion proteins.⁴ Epitope tags provide a method to localize gene products in a variety of cell types, study the topology of proteins and protein complexes, identify associated proteins, and characterize newly identified, low abundance or poorly immunogenic proteins when protein specific antibodies are not available.^{4,5,6} Anti-c-Myc agarose is useful in the purification of expressed c-Myc tagged fusion proteins in bacterial lysates or in transfected cells.

Reagent

Anti-c-Myc Agarose is provided as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data 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 and extended storage, store at 2 °C to 8 °C. **Do not freeze.**

Product Profile

1 ml of settled Anti-c-Myc, Agarose conjugate has a binding capacity of at least 2 nmole of c-Myc tagged fusion protein (i.e. 0.2 mg of an ~120 kDa protein).

At least 1.5 nmoles of a c-Myc tagged fusion protein can be eluted at high pH from 1 ml of settled Anti-c-Myc, Agarose conjugate.

Note: Binding capacity and elution capacity may vary, depending on the characteristics of the c-Myc tagged fusion proteins. For optimal results it is recommended to try different elution buffers.

Procedures

Purification of c-Myc Fusion proteins

- Highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity.
 - Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column.
 - Perform all steps at room temperature.
- A. Column Set Up
1. Place the empty chromatography column on a firm support.
 2. Rinse the column with PBS (Product No. D 8537).
 3. Allow the buffer to drain from the column and leave residual PBS in the column to aid in packing the Anti c-Myc Agarose conjugate.

B. Packing the Column

1. Thoroughly suspend the vial of c-Myc Agarose conjugate to make a uniform suspension of the beads.
2. Immediately transfer the desired volume to the column. Allow the agarose bed to settle.
Do not let the agarose bed dry.

C. Washing the Column

Wash the resin with three sequential 5 ml aliquots of 0.1 M ammonium hydroxide, pH 11 to 12, followed by three sequential 5 ml aliquots of PBS. Avoid disturbing the agarose bed while loading.

D. Binding c-Myc tagged fusion protein to the column

1. Load the lysate on the column under gravity flow. Note: Depending upon the fusion protein and the flow rate, not all of the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator for about 1 hour may improve the binding efficiency.
2. Collect the "flow through" of unbound protein.
3. Wash the column with PBS until $OD_{280} = 0.01$.

E. Elution of c-Myc tagged fusion proteins

Elute the bound c-Myc tagged fusion protein from the column with 10 x 1 ml aliquots of 0.1 M ammonium hydroxide at pH 11 to 12 into vials containing 30 to 50 μ l of 1 N acetic acid for neutralization.

Note: The column may lose activity after prolonged exposure to low pH.

Procedure for Immunoprecipitation of c-Myc tagged fusion proteins

This procedure can be performed in 1.5 ml microcentrifuge tubes or in spin columns (e.g. Product No. S 3563).

1. Add 40 to 100 μ l of the 1:1 suspension of the Anti-c-Myc Agarose conjugate to a microcentrifuge tube or a spin-column.

2. Allow the resin to settle by a short spin. Discard the liquid.
3. Wash the resin 5 times with 1 ml PBS (Product No. D 8537).
4. Add clarified bacterial lysate or cell extract to the settled resin. Bring the volume to at least 200 μ l with PBS or RIPA buffer, if needed.
5. Incubate for 1.5 hours on an orbital shaker at room temperature or at 4 °C.
Note: Shaking must be vigorous enough to suspend the resin.
6. Wash the resin 4 times with 1 ml of PBS or lysis buffer (RIPA).
7. After the final wash, aspirate the supernatant and leave ~10 μ l above the beads.
8. Add 20 to 50 μ l 2X SDS sample buffer. Cap the tube or spin column securely and incubate 5 minutes at 95 °C.
9. Vortex then centrifuge for 5 seconds. Load the supernatant, carefully avoiding the agarose, into a gel lane and analyze by SDS-PAGE.
10. Detection of the c-Myc-tagged fusion protein is determined by Immunoblotting, using monoclonal Anti-c-Myc (Product No. M 5546).

References

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