Product Information

Anti-c-Myc Agarose Affinity Gel

Antibody produced in rabbit; affinity isolated antibody

A7470

Product Description

The human c-myc proto-oncogene is the human cellular homolog of the avian v-myc gene found in several leukemogenic retroviruses.[1-3] 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.[4-6] Anti-c-Myc Agarose Affinity Gel is useful in the purification of expressed c-Myc tagged fusion proteins in bacterial lysates or in transfected cells.

Anti-c-Myc Agarose Affinity Gel is prepared with an affinity purified anti-c-Myc antibody, produced in rabbit, coupled to cyanogen bromide-activated agarose. The purified antibody is immobilized at 1.0-1.5 mg antibody per mL agarose. Anti-c-Myc was produced 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.

Reagent

This product is supplied as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do not freeze.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product Profile

Binding Capacity

1 mL of settled Anti-c-Myc Agarose Affinity Gel has a binding capacity of ≥ 10 nmole of c-Myc-tagged fusion protein.

Elution Capacity

≥ 7 nmoles of a c-Myc tagged fusion protein can be eluted at high pH from 1 mL of settled Anti-c-Myc Agarose Affinity Gel.

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-tagged 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.

Column Set Up

1. Place the empty chromatography column on a firm support.
2. Rinse the column with PBS (such as D8537).
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 Affinity Gel.

Packing the Column

1. Thoroughly suspend the vial of c-Myc Agarose Affinity Gel 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.

Washing the Column

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

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 OD280 ≤ 0.01.

Elution of c-Myc tagged fusion proteins

Elute the bound c-Myc tagged fusion protein from the column with 10 × 1 mL aliquots of 0.1 M ammonium hydroxide at pH 11 to 12 into vials containing 30 to 50 µL 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.

1. Add 40 to 100 µL of the 1:1 suspension of the Anti-c-Myc Agarose Affinity Gel 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 (such as D8537).
4. Add clarified bacterial lysate or cell extract to the settled resin. Bring the volume to at least 200 µL 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 mL above the beads.
8. Add 20-50 µL 2X SDS Sample Buffer. Cap the tube or spin column securely. Incubate for 5 minutes at 95 °C.
9. Vortex then centrifuge for 5 seconds. Load the supernatant, carefully avoiding the agarose, into a gel lane. Analyze by SDS-PAGE.
10. Detection of the c-Myc-tagged fusion protein is determined by Immunoblotting, using Monoclonal Anti-c-Myc (M5546).

References

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