# Anti-c-Myc Tag (9E10) Affinity Gel Protocol V.1

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## Abstract

**Catalog Number:** 658502

**Storage Temperature:** 2°C-8°C

**Product Description** Anti-c-Myc Tag (9E10) Affinity Gel consists of anti-c-myc monoclonal antibody (clone 9E10), covalently immobilized onto 6% high density glyoxal agarose beads. The affinity resin can be used in affinity purifying and immunoprecipitation of c-Myc-tagged fusion protein.

**Binding Specificity** Mouse monoclonal antibody 9E10 recognizes the c-myc epitope N-EQKLSEEDL-C and is purified through Protein G chromatography. The antibody conjugated affinity resin can bind to epitope at N-terminal, C-terminal, and internal locations of a fusion protein. The binding capacity is greater than 0.5mg per ml of c-myc agarose resin.

**Reagent** Anti-c-Myc Agarose Affinity Gel is supplied as a 50% suspension in phosphate-buffered saline, pH 7.2, containing 0.09% sodium azide.

**Precautions and Disclaimer** This product is for research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage/Stability** Anti-c-Myc Agarose Affinity Gel should be stored between 2°C and 8°C for maximum stability. The unopened product is stable for one year when stored as indicated. After use, the resin should be regenerated and stored in TBS or PBS buffer (pH 7.2) containing 0.09% sodium azide.

## Attachments

FILE

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88KB

## Guidelines

| | |
|---|---|
| ▪ Column equilibration buffer: 1xPBS/0.5 M NaCl<br>▪ High salt wash buffer: 50mM Tris 8.0/2M NaCl<br>▪ Elution buffer: 200mM Glycine/150mM NaCl, pH 2.2<br>▪ Neutralization buffer: 2M Tris pH 8.0 | ▪ Other buffers needed: Bacteria breakage buffer (would depend on the laboratory preference).<br>Suggested buffer: 50 mM Tris PH 8.0/150mM NaCl, 1mM EDTA, 1mM DTT, 1mM PMSF and 1mg/ml lysozyme.<br>▪ Mammalian breakage buffer (would depend on the laboratory preference).<br>Suggested buffer: 1xPBS or 50mM Tris 7.4/ 150mM NaCl supplemented with 1% NP40, 0.25% Na-deoxycholate, 1mM EDTA with 1mM PMSF and protease inhibitor cocktail. |

## Part I. Cell Lysate Preparation: Purifying c-Myc fusion proteins from crude E. coli extracts

1 Grow the bacteria cells under the condition that induce production of c-Myc fusion proteins.

2 Harvest the cells by centrifugation at 10,000 rpm for 20 minutes at 4°C.
00:20:00

3 Decant the medium and freeze the cell pellet at -20°C for enhanced cell lysis. At this step, cell pellet can be stored at -80°C for up to 2 years.

4 Thaw the cell pellet at 37°C. Mix pellet in appropriate amount of Breakage Buffer then proceed to lyse cells by sonication. Nuclease treatment is optional.

5 Centrifuge at 10,000 rpm for 30 minutes.

**Note:** If the fusion protein is soluble, discard pellet of cell debris. Transfer the supernatant into a clean container and filter with a 0.45 or 0.22µm filter to remove particulate matter. Supernatant is now ready for c-Myc column purification.

If the fusion protein is in insoluble form, solublize the inclusion body after centrifugation by denaturant, refold the protein properly, change buffer into PBS (pH 7.2) then filter the refolded protein sample by a 0.45 or 0.22µm filter to be ready for purification.
00:30:00

## Part I. Cell Lysate Preparation: Purifying c-Myc fusion proteins from mammalian cells

6 Adherent cells: trypsinize cells. Suspension cells: skip this step.

7 Collect enough mammalian cells by centrifugation.

8 Properly lyse the cell pellet.

9 Centrifuge to remove the cell debris.

10 Supernatant should be filtered by a 0.45 or 0.22µm filter to prepare for c-Myc column purification.

## Part II. Resin Preparation

11 Thoroughly suspend the resin by gentle inversion until the Anti-c-Myc-Agarose Affinity Gel is a uniform suspension of gel beads. Transfer an appropriate aliquot of gel slurry into a clean chromatography column and allow the gel bed to drain by gravity. Do not let the gel bed run dry.

12 Equilibrate the column by two sequential column volumes of PBS containing 0.5M NaCl.

## Part III. C-Myc Fusion Protein Purification

13 For smaller volume of cell lysate (100~200ml), pass the cell lysate through the affinity gel column once or multiple times to enhance the binding efficiency. For large volume of cell lysate, add the equilibrated c-Myc resin to the cell lysate and mix gently at room temperature or 4°C for 3 to 5 hours using an overhead mixing device or a stirrer. Then load sample mixture onto a clean chromatography column, allow the resin to settle and drain by gravity. Collect the flowthrough and test on a SDS-gel to confirm the complete binding of fusion protein to the c-Myc resin. (For very small amount of protein extract (1~2ml), add equilibrated c-Myc resin to the individual tubes with cell extract and gently rock at room temperature or 4°C for 3-5 hours, followed by centrifugation at 2,000 rpm for 2-3 minutes).

14 Wash the column by equilibration buffer. Monitor the flow through by Bradford assay. Wash appropriate amount of equilibration buffer until the Bradford assay indicates baseline is met.

15 Elute the protein by Acid Elution Buffer. Elute into fractions containing appropriate amount of 2M Tris pH 8.0 to immediately neutralize the elute (the exact amount of 2M Tris pH 8.0 should be determined before experiment by testing the pH value of various volume combination of acid elution buffer and 2M Tris pH 8.0). Monitor the eluted protein concentration in the sequential elute aliquots by Bradford Assay or optical absorbance at 280nm. Terminate elution step when baseline is met. Alternatively, the fusion protein can be eluted by adding c-Myc peptide to compete the binding to the affinity resin.

16 Run a SDS-gel for all the elute fractions and combine the ones with desired concentration and purity base on the gel result. Dialyze the protein product into appropriate buffer and stored properly.

## Part IV. C-Myc Affinity Gel Regeneration and Storage

17 Wash the resin with at least five column volume of high-salt buffer to remove any residual protein retained on the resin after elution.

18 Re-equilibrate the resin with at least five column volumes of PBS/0.5M NaCl for immediate usage. If not used immediately, wash the resin by two column volumes of PBS, then store in sufficient amount of PBS/0.09% azide at 4°C. The resin can be used for up to 10 times without any obvious loss of binding capacity