Anti-DYKDDDDK Tag (L5) Affinity Gel Protocol V.2

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Guidelines

Catalog Number: 651501, 651502, 651503

Storage Temperature: 4°C

Product Description: Anti-DYKDDDDK tag (L5) affinity gel is a purified rat IgG2a, κ monoclonal antibody covalently attached to agarose by hydrazide linkage. It is useful for purification or immunoprecipitation of DYKDDDDK-tagged fusion proteins. The binding of antibody to the DYKDDDDK tag is not calcium dependent.

Binding Specificity: Octapeptide (N-DYKDDDDK-C) at N-terminal, Met-N-terminal, C-terminal, and internal locations of a fusion protein. The binding capacity is greater than 0.8mg/ml of L5 agarose resin.

Reagent: Anti-DYKDDDDK tag (L5) affinity gel is supplied as a 50% suspension in phosphate-buffered solution, 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-DYKDDDDK tag (L5) affinity gel should be stored in at 4°C for maximum stability. The unopened product is stable for one year when stored as indicated. After use, the resin should be cleaned and stored in TBS or PBS buffer (pH 7.2) containing 0.09% sodium azide.

Materials

- **Column equilibration buffer:** 1xPBS/0.5M NaCl
- **High salt wash buffer:** 50mM Tris 8.0/2M NaCl
- **Elution buffer:** 200mM Glycine/150mM NaCl, pH 2.2
- **Neutralization buffer:** 2M Tris pH 8.0
- **Other buffers needed:** Bacteria breakage buffer (would depend on the laboratory preference). Suggested buffer: 50 mM Tris PH 8.0/150mM NaCl, 1mM EDTA, 1mM DTT, 1mM PMSF and 1mg/ml lysozyme.
- **Mammalian breakage buffer** (would depend on the laboratory preference). 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 - A. Purifying DYKDDDDK-tag fusion proteins from crude E.coli extracts

1 Grow the bacteria cells under the condition that induce production of DYKDDDDK-tag fusion proteins.

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

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 in appropriate amount of Breakage Buffer.

5 Sonicate mixture to completely lyse cells.

6 Centrifuge at 10,000 rpm for 30 minutes. Transfer supernatant into a clean container and filtered with a 0.45 or 0.22µm filter.

7 If the DYKDDDDK-tag fusion protein is in soluble form, this supernatant is ready for L5 column purification. If the DYKDDDDK-tag fusion protein is in an insoluble form, solubilize the inclusion body after centrifugation step (Step 6), refold the protein properly, change buffer into PBS (pH 7.2) and filter the protein sample by a 0.45 or 0.22µm filter to be ready for L5 purification.

Part I. Cell Lysate Preparation: B. Purifying DYKDDDDK-tag fusion proteins from mammalian cells

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

9 Collect enough mammalian cells by centrifugation.

10 Properly lyse the cell pellet.

11 Centrifuge to remove the cell debris.

12 Supernatant should be filtered by a 0.45 or 0.22µm filter to prepare for L5 purification.

Part II. Resin Preparation

13 Thoroughly suspend the resin by gentle inversion until the bottle of AntiDYKDDDDK-tag (L5) affinity gel is a uniform suspension of gel beads. Transfer an appropriate aliquot into a clean chromatography column and allow the gel bed to drain naturally. Do not let the gel bed run dry.

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

Part III. L5 column purification

15 For smaller volume of cell lysate (100~200ml), pass the cell lysate through the DYKDDDDK-tag (L5) affinity gel column once or multiple times to enhance the binding efficiency. For large volume of cell lysate, add the equilibrated L5 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 platform shaker. Then load sample mixture onto a clean chromatography column, allow the resin to settle and drain naturally. Collect the flow through and test on a SDS-gel to confirm the complete binding of DYKDDDDK-tag fusion protein to the L5 resin. (For very small amount of protein extract (1~2ml), add equilibrated L5 resin (binding capacity is >0.8mg/ml) to the individual tubes and gently rock at room temperature or 4°C for 3-5 hours, followed by centrifugation at 2,000 rpm for 2-3 minutes).

16 Wash the column by equilibration buffer (PBS/0.5M NaCl). Monitor the flow through by BFA reaction. Wash appropriate amount until the BFA test becomes baseline.

17 Elute the protein by Acid Elution Buffer (200mM Glycine/150mM NaCl, pH 2.2). Elute into aliquots 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 combination of acid elution buffer and 2M Tris pH 8.0). Monitor the protein concentration in the sequential elute aliquots by BFA reaction or A280. Terminate elution step when protein is completely eluted. Alternatively, the fusion protein can be eluted by adding DYKDDDDK peptide to compete the binding to the L5 resin.

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

Part IV. L5 Resin Regeneration and Storage

19 Wash the resin with at least five column volume of high-salt buffer (50mM Tris 8.0/2M NaCl) to remove any residual protein retained on the resin.

20 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.