Vector® Fusion-Aid™ - HA Kit

Catalog No. MB-0734

Instructions for Use:

1. Snap off the tip of a spin column and add 0.2 ml of agarose gel slurry into the top part of the column. Place the column into a collection tube and centrifuge for 1 minute in a microcentrifuge.[a]
2. Remove the spin column from the collection tube and discard the flow-through buffer. Place the spin column back into the tube.
3. Wash the gel by adding 0.4 ml of PBS (10 mM phosphate, 150 mM NaCl, pH 7.5) to the spin column. Centrifuge for 1 minute in a microcentrifuge.
4. Remove the spin column from the collection tube and again discard the flow-through buffer. Place the spin column back into the tube.
5. Repeat Steps 3 and 4 two times.
6. Add 0.2-0.5 ml of the sample containing the protein to be purified[b] to the gel in the spin column and incubate for 10 minutes. (For unstable target proteins, incubation at 4 °C overnight is recommended.)
7. Centrifuge for 1 minute in a microcentrifuge. Repeat Steps 6 and 7 until the entire sample has been applied, transferring the pooled flow-through solution into a microcentrifuge tube to be analyzed later.
8. To wash the gel prior to elution, add 0.4 ml of PBS to the gel to allow complete suspension.
9. Centrifuge for 1 minute in a microcentrifuge. Discard the flow-through buffer.
10. Repeat Steps 8 and 9 three times.
11. Transfer the spin column to a new microcentrifuge tube. Add 0.1 ml of elution buffer (PBS with 2% SDS) to the gel in the spin column. Incubate for 5 minutes at 85 °C. [c] Centrifuge the tube for 1 minute in a microcentrifuge.

NOTES:

a. The gel can withstand forces up to 5,000x g without collapsing.

b. The sample should be in PBS or buffer with neutral pH. The flow-through should be saved and analyzed with the eluent to ensure complete isolation of the fusion protein.

c. Elution at 85 °C can denature the protein and should not be done if a functional protein is needed. For milder conditions, elution can be performed at room temperature using the same elution solution. To maximize recovery, up to three elutions may be required. However, the efficiency of such elution may be lower than when performed at a higher temperature. Individual eluate fractions can be analyzed by SDS-PAGE, western blotting or other methods and positive fractions combined.

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