



## Vector® Fusion-Aid™ - HA Kit

Catalog No. MB-0734

This Fusion-Aid™ kit is designed to isolate fusion proteins containing HA-tag. This kit contains an affinity-purified antibody to HA-tag coupled through a stable hydrophilic spacer arm to crosslinked 4% agarose beads. Spin columns and collection tubes are included for convenience. For optimal results it is recommended that the protocol on the reverse side of this specification sheet be followed.

Product AGAROSE ANTI-HA  
\*\*AFFINITY PURIFIED\*\*

Amount 0.5 ml of settled gel (1:1 v/v slurry)

Lot No. M1016

Produced in goat

Suspension Solution 10 mM phosphate, 0.15 M NaCl, pH 7.5, 0.08% sodium azide

Antibody Concentration 2.0 mg/ml of settled gel

Binding Capacity > 0.4 mg HA tagged  $\beta$ -galactosidase (120kD)/ml gel\*

Storage Conditions Refrigerate - DO NOT FREEZE

### Other Remarks:

\* Actual binding capacity may vary depending on the size of the fusion protein.

### Related Products

	<u>Cat. No.</u>	<u>Unit Size</u>
Biotinylated Anti-HA, affinity purified, made in goat	BA-0704	0.25 mg
VECTASTAIN® ABC-AmP™ Reagent (Standard Kit)	AK-6000	1 kit
DuoLuX™ Chemiluminescent/Fluorescent		
Substrate for Alkaline Phosphatase	SK-6605	100 ml
BCIP/NBT Substrate Kit	SK-5400	1 kit
10x Casein Solution	SP-5020	250 ml
Biotinylated Protein Molecular Weight Markers	SP-1400	50 blots

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## 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.<sup>a</sup>
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 and incubate for 1 minute with gentle agitation. 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<sup>b</sup> to the gel in the spin column and incubate for 10 minutes with gentle agitation. (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, and incubate for 1 minute with gentle agitation.
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 with gentle agitation. Centrifuge the tube for 1 minute in a microcentrifuge.<sup>c</sup>

## **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. 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. Individual eluate fractions can be analyzed by SDS-PAGE, western blotting or other methods and positive fractions combined.