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A Geno Technology, Inc. (USA) brand name

Immobilized Monomeric Avidin Kit

(Cat. # 786-597)

INTRODUCTION

G-Biosciences Immobilized Monomeric Avidin Resin is designed for the simple affinity chromatography purifications of proteins, antibodies and other molecules with a biotin tag. The resin consists of monomeric subunits of avidin covalently coupled to 6% cross-linked agarose, offering a stable, reusable resin for the purification of biotinylated molecules.

Monomeric avidin offers a distinct advantage over native avidin, a tetrameric molecule, and streptavidin as it has a much lower biotin binding affinity, $K_d=10^{-7}$ as opposed to $K_d=10^{-15}$ for native avidin. This lower binding affinity allows elution of molecules with mild elution buffers (2mM D-Biotin in 1X PBS), as opposed to the strong denaturing buffers (8M Guanidine•HCl, pH 1.5) used with native avidin.

The covalent attachment of monomeric avidin to the agarose ensures no detectable leaching of the avidin during biotin purification and offers a wide tolerance to chemicals. This ensures the column can be reused at least 10 times with no loss of function.

ITEMS SUPPLIED (Cat. # 786-597)

Part. #	Description	Size*
050I-A	Immobilized Monomeric Avidin Column	2ml resin
001J	JAW™ Phosphate Buffered Saline Pack	1
145M-A	Monomeric Avidin Blocking & Elution Buffer	200ml
146M-A	Monomeric Avidin Regeneration Buffer	250ml
410S-B	Stopper, Plastic	1

** Immobilized avidin resin is supplied as a 50% slurry with 0.02% sodium azide as a preservative.*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store refrigerated at 4°C, **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- Biotin Binding Capacity: ≥1.2mg biotinylated BSA/ml resin
- Bead Structure: 4% cross-linked agarose

ADDITIONAL ITEMS

- Biotinylated protein, antibody or other molecules in solution (1-3mg biotinylated protein/ml packed resin)

PREPARATION BEFORE USE

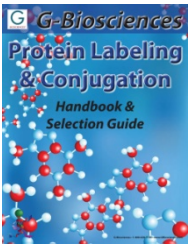
- PBS Wash Buffer: Add the JAW™ Phosphate Buffered Saline (PBS) Pack to 500ml ultrapure water and stir to dissolve. Store buffer at 4°C, for long term storage, sterile filter and supplement with 0.02% sodium azide.

PROTOCOL

1. Allow the column and reagents to equilibrate to room temperature.
2. Briefly, centrifuge the column at 1,000-2,000g for 1-2 minutes to collect the resin in the bottom of the column.
3. Remove top cap and twist off bottom tab. Allow the storage solution to drain from column.
4. Wash the column with 2 x 4ml PBS Wash Buffer.
5. Wash the column with 2 x 4ml Monomeric Avidin Blocking & Elution Buffer.
6. Wash the column with 3 x 4ml Monomeric Avidin Regeneration Buffer.
7. Wash the column with 4ml PBS Wash Buffer.
8. Add the biotinylated antibody/protein/molecule to the column and allow it to enter the resin. Apply 100µl PBS Wash Buffer to ensure the sample fully enters the resin bed. Place a stopper on the bottom of the column and then apply a cap to the top of the column.
   Note: *If the volume of the sample is too large, then add appropriate amount, incubate for 10 minutes, drain column and repeat steps 7. Do not exceed resin's binding capacity.*
9. Incubate the column at room temperature for up to 60 minutes.
   Note: *Binding is only slightly increased with incubation.*
10. Wash the column with 12ml Wash Buffer and monitor absorbance of 2ml fractions at 280nm. When a baseline has been reached the non-bound protein has been removed.
11. Elute the protein by adding 2ml fractions of Monomeric Avidin Blocking & Elution Buffer. Elute with a total volume of at least 12ml. Monitor protein collection with absorbance at 280nm.
    Note: *The Monomeric Avidin Regeneration Buffer may be a better elution buffer for some proteins. If used, neutralize the pH with 0.1 volumes of 1M Tris, pH9 after elution.*
12. Regenerate the column with 4 column volumes of regeneration buffer. For long term storage, wash with 2.5 column volumes of PBS Wash Buffer supplemented with 0.02% sodium azide. Store upright at 4°C.
    Note: *The resin and columns can be regenerated at least 10 times without significant loss of performance.*

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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