Molecular Biology Grade AG® 50W-X8 Cation Exchange Resin

Instruction Manual

BIO-RAD

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Section 1
Introduction

Ethidium bromide is used to visualize DNA and RNA preparations because it intercalates between the bases and fluoresces when irradiated with UV light at 300 nm. Propidium iodide, which also intercalates between bases, is used to increase the separation of superhelical and non-supercoiled DNA in cesium chloride gradients. Molecular Biology Grade AG 50W-X8 cation exchange resin is useful for removing these dyes, to provide a purified plasmid preparation. The resin is certified to be free of endo-and exo-nuclease, and ligase inhibitors. The resin has a high selectivity for the hydrophobic, cationic ethidium and propidium ions and repels the strongly anionic nucleic acid. The fluorescent labeled DNA is passed over a column of the Molecular Biology Grade AG 50W-X8 cation exchange resin, which removes the fluorescent dye from the DNA or RNA. The pure plasmid preparation is then collected in the void volume.

Section 2
Description

AG 50W-X8 cation exchange resin is the backbone resin of the Molecular Biology Grade AG 50W-X8 resin. AG 50W-X8 resin is a strong cation exchanger with sulfonic acid functional groups attached to a styrene divinylbenzene copolymer lattice. The Molecular Biology Grade resin is provided in the sodium form. It has been specially equilibrated in Tris buffer at pH 8.0. The resin is capable of exchanging cations of salts, and of ampholytes on the acidic side of their pI. This resin can be used to extract basic compounds from a solution with a pH at least 1 unit lower than the pK_a of the analyte. The analytes are then eluted with a neutral or basic pH solution.

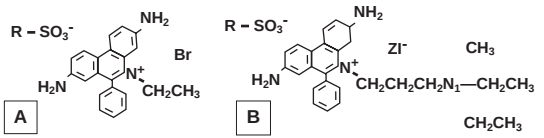

Fig. 1. Removal of (A) ethidium bromide and (B) propidium iodide by AG 50W-X8 cation exchange resin. R is the resin matrix; SO_3^- is the functional group covalently attached to the resin.

Section 3
Instructions for Use

For removing ethidium and propidium salts from plasmids, the resin is slurried in a pH 8.0 Tris buffer and poured into a column. The sample pH is adjusted to pH 8.0. Thus the DNA will be anionic, and ethidium and propidium will be cationic. When the sample is added to the column, the ethidium and propidium are exchanged for sodium ions on the resin. The pure plasmid preparations pass through the column immediately (in the void volume) and the fluorescent dyes are bound to

the resin. Figure 1 shows the removal of (A) ethidium bromide and (B) propidium iodide by Molecular Biology Grade AG 50W-X8 cation exchange resin. R is the resin matrix; SO_3^- is the functional group covalently attached to the resin.

Section 4
Sample Protocol

The following procedure was developed by Rodriquez and Tait to remove propidium iodide from intact plasmids, and is used for DNA concentrated on cesium chloride gradients.[1]

4.1 Material required

Concentrated HCl

A-50 buffer (4 liters):

Trizma base	24 grams (50 mM)
NaCl	117 grams (500 mM)
0.25 M EDTA	16 ml (1 mM)
1 M NaN3	4 ml
Distilled water	3.5 liters

Adjust pH to 8.0 with 8-10 ml concentrated HCl. Add distilled water to 4.0 liters.

500 ml beaker

Test tube rack for Poly-Prep® chromatography columns

Test tubes or other containers to collect DNA

4.2 Protocol

1. Use about 1.5 ml (approximately 1.1 grams) of Molecular Biology Grade AG 50W-X8 resin in a column for each gradient tube. The resin may also be autoclaved in the Poly-Prep chromatography columns, if a sterile preparation is desired.
2. Slurry the resin for each column to be poured in 2.3 volumes of A-50 buffer. Pour all at once into a Poly-Prep column. Do not snap the tip off the column until ready to proceed with Step 3. At this time, the resin and column may be autoclaved.
3. Snap the tip off the column and wash the column with 20 ml of A-50 buffer.

4. Dilute DNA with an equal volume of A-50 buffer and apply the DNA sample to the column. Dilution of the sample is necessary to slow the flow rate and allow complete removal of the propidium or ethidium. Use a stopcock to slow the flow rate if necessary.
5. Add 1 bed column (1.5 ml) of A-50 buffer to wash out any remaining DNA.
6. Check DNA sample with UV to insure that the dye has been removed. If not, remove the top layer of the resin containing the dye, discard, and run the DNA sample over the column again.

Warning: Ethidium and propidium are carcinogenic. The used columns should be disposed of as hazardous waste.

Section 5
Reference

1. Rodriquez, R. L. and Tait, R. C., Recombinant DNA Techniques: An Introduction, Addison-Wesley Publishing Co., Reading, Massachusetts, 153-158 (1983).

Section 6
Product Information

Catalog Number	Mesh Size	Ionic Form	Wet Pkg. Size	Capacity (meq/ml)	Diameter (microns)	Density (Nominal) gm/ml
Molecular Biology Grade AG 50W-X8 Resin						
143-1441	100-200	Sodium	100 g	1.7	106-250	0.80
143-1451	200-400	Sodium	100 g	1.7	63-150	0.80
Columns						
731-1550	**Poly-Prep® Columns**, empty, 50					
731-7005	**Poly-Prep Column Rack**, 1					

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
LIT304 Rev B