

Application

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Rapid Elimination of Ethidium Bromide from Stained DNA Using Rezorian™ A161 Cartridges

Using Rezorian A161 cartridges to remove mutagenic ethidium bromide from stained DNA is an effective and rapid alternative to conventional EtBr removal methods. Hazardous waste is reduced to a small, contained volume, and the need for organic extraction of DNA with butanol is eliminated. (ChromFax: 394022)

Key Words:

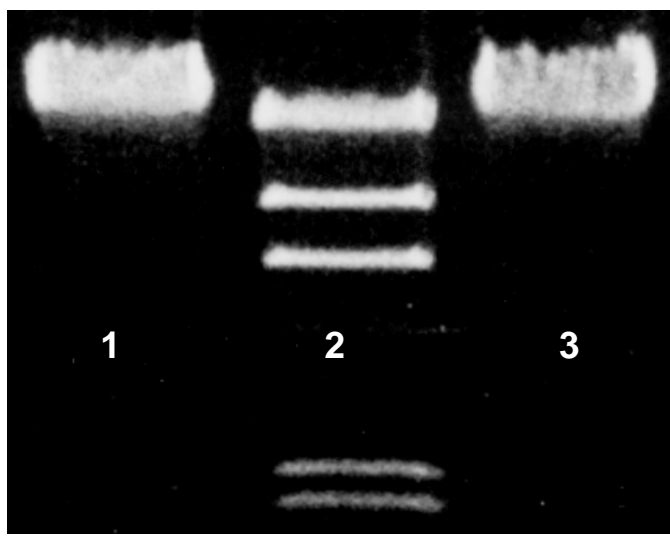
- ethidium bromide ● Rezorian A161 cartridges ● DNA

Purification of plasmid DNA by typical methods carries the risk of human contact with mutagenic ethidium bromide (EtBr). The nucleic acid extracted from the bacterial clone is stained with EtBr and ultracentrifuged in cesium chloride gradient. The stained DNA migrates through the gradient during ultracentrifugation and forms a fluorescent band that is detected by ultraviolet (UV) light. The DNA band is collected and extracted with n-butanol to remove the EtBr (1). Our investigation shows that Rezorian A161 cartridges may be used for removing EtBr from the stained DNA sample during purification, thus eliminating the necessity for organic extraction of the nucleic acid.

Rezorian A161 cartridges effectively remove mutagenic EtBr from large volumes of solution, reducing hazardous waste to a small volume of solid material contained in the cartridge. These cartridges can adsorb 0.5µg/mL from more than 16 liters of solution. As the resin beads in Rezorian cartridges adsorb EtBr, they turn pink in color. Breakthrough is determined by monitoring the absorbance at 275nm or 485nm, or by piggybacking two cartridges. As pink begins to show in the second cartridge, the first cartridge has reached breakthrough.

In our analysis, lambda DNA (25µg/mL) stained with EtBr (100µg/mL) was passed through a 1mL Rezorian A161 cartridge. The EtBr bound to the cartridge, causing the matrix to turn pink in color, while the DNA passed through as a transparent solution. To determine if any DNA was lost following elution from the resin, we electrophoresed the eluted DNA on 0.7% agarose gel in Tris-borate-EDTA buffer. The gel was stained with EtBr and photographed (Figure A). There was no detectable DNA loss following passage through the Rezorian cartridge. The concentration of the eluted DNA was equivalent to that of the control DNA that was not passed through the cartridge (Figure A, lanes 1 and 3). A portion of the eluted DNA was digested with the restriction enzyme *Hind* III (lane 2), indicating that the DNA did not undergo any drastic alterations and that it can be further processed following elimination of the intercalating EtBr molecules.

Figure A. Agarose Gel Electrophoresis of DNA Following Elution from a Rezorian A161 Cartridge



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- Lane 1 — Lambda DNA stained with EtBr (100µg/mL) and elution from a Rezorian A161 cartridge.
- Lane 2 — DNA eluted from Rezorian digested with *Hind* III.
- Lane 3 — Control lambda DNA not passed through a Rezorian cartridge.

The protocol for eliminating EtBr from stained DNA follows:

1. Thoroughly rinse a Rezorian A161 cartridge using 10mL of sterile water introduced from a syringe. Pass air through the cartridge to remove all effluent.
2. Apply EtBr-stained DNA sample onto the cartridge, using a syringe or micropipette.
3. Elute DNA, using water or TE buffer. The DNA elutes immediately.

NOTE: To avoid sample dilution, modify step 3. Pass about 10 column volumes of air through the cartridge, using a syringe, until all DNA is eluted.

The minimum volume of sample that can be applied to the 1mL Rezorian cartridge is 100µL. No buffer or water is required for DNA elution, to avoid sample dilution. Air may be passed through by a syringe in order to recover the sample. Up to 80% of the injected solution was recovered when passing air through the cartridge. More than 90% was recovered when injecting 200µL. The EtBr binds to the matrix and the DNA is eluted in the original buffer. The DNA may also be eluted in water or TE buffer (10mM TrisHCl, 1mM EDTA, pH 8.0).

Ordering Information:

Rezorian A161 Cartridges

Reagent Grade, pk. of 6	
1 mL	57610-U
5 mL	57611

Holder for Rezorian Cartridges

For high pressure applications	
1 mL	57605-U
5 mL	57606

Reference

1. Sambrook, J., E.F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989.

Trademark

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