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If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.

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

C. DebRoy

Rezorian A161 cartridges effectively remove mutagenic ethidium bromide from stained DNA. Hazardous waste is reduced to a small, contained volume, and the need for organic extraction of DNA is eliminated.

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 a cesium chloride gradient. The stained DNA migrates through the gradient during ultracentrifugation and forms a fluorescent band that is detected by ultraviolet light. The DNA band is collected and extracted with n-butanol to remove the EtBr (1).

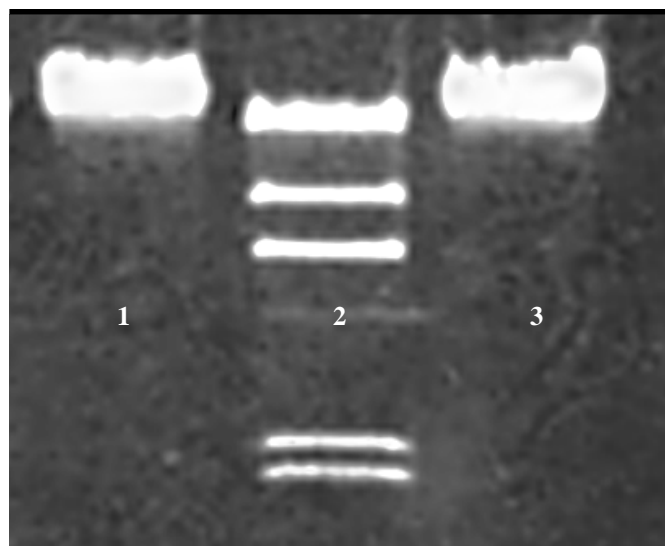
Rezorian A161 cartridges effectively remove EtBr from large volumes of solution, reducing hazardous waste to a small volume of solid material contained in the cartridge (2).

We used Rezorian A161 cartridges to remove EtBr from stained DNA during purification, eliminating the need for organic extraction of the nucleic acid. Lambda DNA (25µg/mL) stained with EtBr (100µg/mL) was passed through a 1 mL Rezorian cartridge. The EtBr bound to the cartridge, causing the matrix to turn pink, 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). No DNA loss was detected 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. 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 1 mL Rezorian cartridge is 100µL. No buffer or water is required for DNA elution. Air may be passed through the cartridge by syringe, to

Figure A. Agarose Gel Electrophoresis of DNA



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Lane 1 — Lambda DNA stained with EtBr (100µg/mL) and eluted from a Rezorian A161 cartridge.

Lane 2 — DNA eluted from a Rezorian cartridge and digested with *Hind* III.

Lane 3 — Control DNA not passed through a Rezorian cartridge.

recover the sample. Up to 80% of the injected solution was recovered in this manner. More than 90% was recovered when injecting 200µL of sample.

Ordering Information:

Rezorian A161 Cartridges, 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

References

1. Sambrook, J., E.F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989.
2. Supelco Reporter, Vol. 11, No. 4 (pp. 6-7); Vol. 13, No. 1 (pp. 20-21). Reference 1 not available from Supelco.

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