

# Application

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## Concentrate Ethidium Bromide Waste Using Polymer-Filled Adsorbent Cartridges

*Rezorian A161 cartridges effectively remove ethidium bromide from large volumes of solution, reducing hazardous waste to a small volume of solid material retained in the cartridge. Reagent grade adsorbent and a flow rate of 1 liter/hour maximize the adsorption capacity of the cartridges. This method of EtBr containment is time- and cost-efficient, and reduces the risk of contact with the mutagen.*

### Key Words:

- ethidium bromide
- Rezorian A161 cartridges

Because it intercalates between the bases in DNA molecules, ethidium bromide (3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide, EtBr) is extensively used as a fluorescent dye in biotechnology research. The characteristics that make EtBr a useful research tool, however, also make the molecule a dangerous mutagen. Formerly, biotechnologists treated ethidium bromide solutions with chlorine bleach in attempts to make the material safe for disposal. While this treatment eliminates the fluorescent character of EtBr, it does not affect the molecule's mutagenicity (1). Consequently, solutions containing EtBr must be disposed of as hazardous waste – a costly venture.

Rezorian™ A161 analytical cartridges selectively remove hydrophobic substances from biological sample solutions, making these cartridges useful for surfactant removal (e.g., Triton® X-100), protein concentration and purification, and sample desalting. They effectively remove ethidium bromide from large volumes of aqueous solutions, eliminating extensive dialysis steps and reducing EtBr waste to a small volume of solid material retained within the cartridge. Once scanned to confirm the removal of EtBr, solutions passed through a Rezorian A161 cartridge can, if local regulations permit, be disposed of in the laboratory sink. **Cartridges containing EtBr should be disposed of according to your regulatory compliance and/or waste hauler agreement.**

Rezorian A161 cartridges contain a hydrophobic divinylbenzene polymer resin (Table 1). The resin is resistant to most chemical solvents, is autoclavable, and functions at pH 0-14. The cartridges, which have luer lock fittings, can be used either in line or in conjunction with syringes. Two 1 mL cartridges connected in series have a total void volume of  $800 \pm 20 \mu\text{L}$ . The cartridges have been tested to back pressures of 70psi (4.9kg/cm<sup>2</sup>).

**Table 1. Characteristics of Rezorian A161 Cartridges**

Bed Volume (mL)	Bed Height (cm)	Cartridge Dimensions (cm)			Void Volume (mL)
		OD	ID	Length (tip-to-tip)	
1	1.8	1.1	0.9	4.5	0.127±0.020
5	4.4	1.4	1.3	7.2	1.6±0.2

### Resin Characteristics

<b>Particle Description:</b>	spherical, divinylbenzene
<b>Mean Particle Size:</b>	120µm
<b>Mean Pore Size:</b>	110-175Å
<b>Surface Area:</b>	800-950m <sup>2</sup> /g

Reagent grade and technical grade resins were investigated for effectiveness as adsorbents for EtBr. Rezorian A161 cartridges containing the resins were rinsed with 60mL of deionized, distilled water, then a 0.05µg/mL or 0.5µg/mL solution of EtBr in deionized water was applied onto a cartridge via a 30mL syringe (Figure A) or a peristaltic pump (Figure B). Absorbance equal to or greater than 0.01 at 285nm was used as the indicator of EtBr breakthrough.

**Figure A. Adsorbing Ethidium Bromide on Rezorian A161 Cartridges — Syringe Method**



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**Figure B. Adsorbing Ethidium Bromide on Rezorian A161 Cartridges — Pump Method**



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Five milliliter Rezorian A161 cartridges containing reagent grade resin adsorbed the EtBr from more than 16 liters of 0.5µg/mL solution without breakthrough; 1mL cartridges adsorbed EtBr from more than 3 liters of solution without breakthrough (Table 2). At this EtBr concentration, flow through the cartridges slowed dramatically long before breakthrough occurred, giving the user ample indication that the cartridge should be replaced. At the lower (0.05µg/mL) concentration, EtBr did not appear in the eluate until the entire bed of reagent-grade resin was stained pink by the adsorbed compound, indicating the mutagen is strongly bound to the adsorbent. Introduction of 0.05µg/mL EtBr solutions into a 5mL cartridge containing technical grade material led to breakthrough at less than 4 liters, when the solution was applied with a peristaltic pump (Table 3). A faster flow rate increased the breakthrough volume, probably because impurities were flushed from the technical grade resin. Similar effects were not seen with the 1 mL cartridges because the smaller bed is packed more tightly, giving it different flow characteristics.

**If EDTA is present in the buffer system, the pH must be dropped to ≤4.0 before the material is applied to a Rezorian A161 cartridge. Unless it is protonated, the EDTA will complex with the EtBr, reducing the capacity of the cartridge.**

**Table 2. Ethidium Bromide Breakthrough on Reagent Grade Adsorbent\***

Bed Volume (mL)	EtBr Concentration** (µg/mL)	Breakthrough Volume (liters)
1	0.5	>3.5
5	0.5	>16.5
5	0.05	>40

\* Flow rate = 1 liter/hour; flow rate decreases long before breakthrough is reached.

\*\* 0.5µg/mL is a typical working concentration of EtBr.

**Table 3. Ethidium Bromide Breakthrough on Technical Grade Adsorbent\***

Bed Volume (mL)	Flow Rate (liters/hour)	Breakthrough Volume (liters)
1	0.315	3.5
1	0.582	2.5
5	1	3.5 ±0.5
5	2	>9

\* EtBr concentration = 0.05µg/mL.

The use of Rezorian A161 cartridges to remove ethidium bromide from solution offers time- and cost-efficient containment of this material, with reduced risk of contact. Batchwise adsorption methods require twenty hours or more before the solution is deactivated, and liquid waste disposal is far more expensive. With 5mL Rezorian A161 cartridges, waste is reduced to a small quantity of confined solid material, as opposed to liters of liquid waste. Aliquot volume and concentration are not critical when adsorbing small molecules, such as ethidium bromide (MW 314), antibiotics, and drugs on Rezorian A161 cartridges. Total available capacity, not concentration or aliquot size, determines breakthrough. **Reagent grade adsorbent and a relatively high flow rate (1 liter/hour) offer the greatest capacity for ethidium bromide or other small molecules.**

### Ordering Information:

Description	Cat. No.
<b>Rezorian A161 Cartridges (pk. of 6)</b>	
1 mL	57610-U
5 mL	57611

### Literature Survey

*Applications for Amberlite®/Duolite® Adsorbent Resins, 1987-1991*

T412139

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 814-359-5468) for expert answers to your questions.

### Reference

- Lunn, G. and E. Sansone, *Anal. Biochem.*, **162**: 453-458 (1987).  
Reference not available from Supelco.

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Note 1

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