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ProductInformation

GenElute™ Minus EtBr Spin Columns

Product Code **5-6501**
Technical Bulletin Code MB-730

TECHNICAL BULLETIN

Product Description
The GenElute™ Minus EtBr Spin Columns provide a rapid and simple method for recovering ethidium bromide (EtBr)-free DNA (100 bp to 10 kb) from stained agarose gels or EtBr containing solutions. The DNA band is excised from an agarose gel and loaded into the spin column. Embedded within the base of the column are a series of membranes and filters that hold agarose and ethidium bromide back while allowing DNA to selectively pass through the column into a collection tube during a 10 minute centrifugation. GenElute Minus EtBr Spin Columns eliminate the need for silica-based resins, DEAE, or toxic organic solvents such as phenol and chloroform. There is no melting, electroelution, or enzymatic digestion of the agarose gel. More than 95% of the ethidium bromide is typically removed from the agarose.

Typical recovery is 30 to 35% for 100 bp to 10 kb DNA fragments. Recovery decreases with increasing fragment size. Optimal recovery can be obtained with gel slices weighing less than 100 mg. A 40 mg gel slice from a 1.0% gel will yield an eluate volume of approximately 40 µl. A 90 mg gel slice from a 1.0% gel will yield an eluate volume of approximately 65 µl. The maximum gel slice weight is approximately 200 mg. The purified DNA can be used in most downstream applications such as ligation, PCR[†], restriction endonuclease digestion, labeling and hybridization.

Materials Provided (70 purifications)	**Product Code**
GenElute EtBr Minus Spin Columns, 70 each	G 2166
Collection Tubes, 2 ml, 140 each	T 7813

Storage
Store the product at room temperature.

Equipment and Reagents Required But Not Provided
- Agarose, Product Code A 9539
- 1X TE (Tris-EDTA) buffer, prepared from 100X TE buffer, Product Code T 9285, or molecular biology reagent water, Product Code W 4502
- Microcentrifuge
- UV light box (transilluminator)
- Razor blade or scalpel

Precautions and Disclaimers
The GenElute Spin Columns are for laboratory use only. Not for drug, household or other uses. None of the components that make up the GenElute Spin Columns are harmful. However, ethidium bromide, which is commonly used to visualize DNA after gel electrophoresis, is a powerful mutagen and moderately toxic. Gloves, safety glasses, and suitable protective clothing are recommended when handling reagents and columns that contain this dye. See the Material Safety Data Sheet (MSDS) for ethidium bromide.

Procedure
All steps are carried out at room temperature.

1. Place the GenElute Minus EtBr Spin Column into a collection tube.

2. Pre-wash the spin column by adding 100 µl of 1X TE (10 mM Tris, pH 8.0, with 1 mM EDTA) or water to the spin column.

3. Cap and centrifuge the spin column at maximum speed (12,000–16,000 x *g*) for 5-10 seconds.

   Note: The pre-washed column should not be allowed to dry. Avoid pre-washing too early before use or centrifuging the pre-wash solution more than 10 seconds.

4. Discard the wash eluate or transfer the spin column to a fresh collection tube.

5. Excise the band of interest from the agarose gel and load the gel slice onto the pre-washed column. For optimal results, trim the gel slice as close as possible to the DNA band. This eliminates the processing of excess agarose and keeps the DNA

more concentrated. See the Product Description Section for information on the maximum gel slice weight. In addition, macerating the gel slice prior to loading it into the spin column will greatly enhance the recovery of the DNA. Gel slices can be ground in a microcentrifuge tube with a pestle, chopped into small particles with a razor blade or scalpel, or passed through a syringe. Breaking up the agarose into smaller pieces allows for more efficient release of the DNA, dramatically improving its recovery.

6. Centrifuge the spin column at maximum speed for 10 minutes. The purified DNA is in the collection tube and is ready to use. The DNA can be stored at 2-8 °C or –20 °C.

7. Optional DNA precipitation procedure:
   a. To one volume of the recovered DNA solution, add 0.1 volumes of 3 M sodium acetate, pH 5.2.
   b. Add 2.5 volumes of absolute ethanol. Incubate at room temperature for 2 hours.
   c. Centrifuge for 15 minutes at maximum speed.
   d. Discard the supernatant.
   e. Wash the DNA pellet with 70% ethanol.
   f. Resuspend the DNA pellet in water or TE buffer.

   Note**:** To avoid a 2 hour incubation, precipitate the DNA using GenElute™ LPA, Product Code 5-6575. Follow the instructions included with the product.

Troubleshooting Guide

Problem	Reason	Solution
Poor or low DNA recovery	Centrifuge speed is too slow.	Make certain that the maximum centrifuge speed is between 12,000 and 16,000 x *g*.
	Insufficient quantity of DNA in gel	Increase the initial DNA quantity loaded onto the gel.
	Agarose concentration in gel is too high.	If possible, reduce the agarose gel percentage.
Purified DNA is too dilute.	Eluate volume is too large.	• Precipitate the DNA (Step 7 of the procedure). • Concentrate the purified DNA in a rotary evaporator. • Trim the gel slice as close to the band as possible.
	Many restriction enzymes are extremely sensitive to salt concentration. Gel and running buffers may interfere with enzyme activity.	• Dilute or dialyze away any salts in the eluate. • Precipitate the DNA (Step 7 of the procedure).
	Boric acid and EDTA in TBE buffer may inhibit enzymatic reactions.	• Dilute out or completely eliminate these two chemicals from the protocol. Use TA buffer (40 mM Tris-acetate, pH 8.3) instead of TBE or TAE buffer, and pre-wash the spin columns with water instead of TE buffer. • Precipitate the DNA (Step 7 of the procedure).
A second upper band appears when purifying covalently closed supercoiled DNA plasmid.	The spin columns are designed to purify <u>linear</u> DNA fragments. Purifying supercoiled DNA will result in nicking. The upper band represents nicked DNA.	Use GenElute™ Plasmid Miniprep, Midiprep, or Maxiprep Kits designed specifically for plasmid isolation.
The purified DNA is still fluorescent.	GenElute Minus EtBr spin columns may not completely remove EtBr intercalated between stacked DNA base pairs. If the purified DNA eluate is too concentrated, either from excess DNA or from a very low eluate volume, the sample may fluoresce.	If complete ethidium bromide removal is necessary, precipitate the recovered DNA with ethanol.

Related Products	Product Codes	Related Products	Product Codes
TBE, 5X concentrate	T 6400	GenElute™ Plasmid Miniprep kits	PLN-10
TAE, 10X concentrate	T 9650		PLN-70
Sodium acetate buffer solution, 3 M, pH 5.2	S 7899		PLN-350
		GenElute™ Plasmid Midiprep kits	PLD-35
GenElute™ LPA	5-6575		PLD-140
GenElute™ Agarose spin columns	5-6500	GenElute™ Plasmid Maxiprep kits	PLX-15
Ethanol, absolute, for molecular biology	E 7023		PLX-70
Ethidium Bromide, 10 mg/ml solution	E 1510	UV Transilluminator	Z36,366-9

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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