GenElute™ Gel Purification Kit

Product No. **GE-PUR**
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ProductInformation

TECHNICAL BULLETIN

Product Description

The GenElute™ Gel Purification Kit is designed for the rapid purification of DNA of 50 bp to 14 kb from standard or low-melting agarose gels in TAE or TBE buffer. Up to 80% of the DNA in a slice of agarose can be isolated using this procedure.

The GenElute Gel Purification Kit combines silica-based membrane technology and the convenience of a spin column format. DNA fragments of interest are extracted from slices of an agarose gel by solubilizing the gel. The Gel Solubilization Solution contains sodium perchlorate, which can dissolve an agarose gel slice from gels run in either TBE or TAE buffer. DNA fragments selectively adsorb onto the silica membrane in the presence of the chaotrope; contaminants are removed by a simple spin-wash. Finally, the bound DNA is eluted in Tris buffer. The isolated DNA is suitable for a variety of downstream applications, such as automated DNA sequencing, PCR‡, restriction digestion, cloning and labeling.

Components Sufficient for 70 purifications	**Product No.**	**Quantity**
Gel Solubilization Solution (GSS)	G6290	70 ml
Wash Solution Concentrate (WS)	W1015	16 ml
Elution Solution (ES; 10 mM Tris-HCl, pH 8.0)	E8276	7 ml
GenElute™ Miniprep Binding Columns in Tubes	G6415	70 each
Collection Tubes, 2 ml	T7813	70 each

Equipment and Reagents Required But Not Provided
(Sigma Product numbers have been given where appropriate)

- Cutting tools for gel (scalpel or razor blade)
- Pipette, with tips
- Water bath or heating block at 50-60°C
- Ethanol (95-100%), Product No. E7148 or E7023
- Microcentrifuge and tubes
- Water, Molecular Biology Grade, Product No. W4502

Precautions and Disclaimer

The GenElute Gel Purification kit is for laboratory use only. Not for drug, household or other uses. Gel Solubilization Solution contains sodium perchlorate, which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling this solution or any reagents provided with the kit. See the Material Safety Data Sheet.

Storage

Store the kit at room temperature.

Preparation Instructions

Wash Solution (WS): Dilute the entire 16 ml of the Wash Solution Concentrate (WS) with 64 ml of 95-100% ethanol prior to initial use. After each use, tightly cap the diluted WS to prevent the evaporation of ethanol.

Procedure

1. **Excise band.** Excise the DNA fragment of interest from the agarose gel with a clean, sharp scalpel or razor blade. Minimize the size of the gel slice by trimming excess agarose gel.

   Note: When initially running the gel, use fresh buffer. Repeatedly used electrophoresis buffer will reduce DNA recovery efficiency.

2. **Weigh gel.** Weigh the gel slice in a sterile microcentrifuge tube.

3. **Solubilize gel.** Add Gel Solubilization Solution to the gel at a 3:1 ratio. For example, for every 100 mg of agarose gel use 300 µl of Gel Solubilization Solution. Incubate the gel mixture at 50-60°C for 10 minutes, or until the gel mixture is completely dissolved. Vortex briefly every 2-3 minutes during incubation to help the gel dissolve.

Note: For a >2% agarose gel, if a 3:1 ratio of solution to gel does not adequately dissolve the gel, increase the ratio to 6:1.

4. **Bind DNA.** Load the entire DNA sample onto the GenElute Miniprep binding column that is assembled in a 2 ml collection tube. Centrifuge at 11,000 X g for 1 minute. Discard the flow-through.

   Note: If the volume of the gel mixture is >800 µl, load the sample onto the column in two portions. The binding column for this kit has a blue o-ring (not to be confused with other GenElute kits).

5. **Wash column.** Add 750 µl of Wash Solution (WS) to the GenElute Miniprep binding column, and centrifuge at 11,000 X g for 1 minute. Discard the flow-through, but retain the collection tube. Centrifuge again at 11,000 X g for 2 minutes without any additional wash solution to remove excess ethanol.

   Note: Prior to first time use, be sure to add ethanol to the WS. See Preparation Instructions.

6. **Elute DNA.** Transfer the GenElute Miniprep binding column to a fresh collection tube. Add 50 µl of Elution Solution (ES) to the column. Centrifuge at 11,000 X g for 1 minute.

   Note: To increase DNA concentration, use 30 µl (minimum) of Elution Solution to elute DNA from column. Repeat elution with the eluate from the first elution to improve recovery.

Troubleshooting Guide

Problem	Reason	Solution
Poor or low recovery	Ratio of Gel Solubilization Solution to gel is incorrect	Use ratio of 3:1, volume (ml) of GSS to weight (g) of gel fragment. For agarose gels >2%, use a ratio of 6:1.
	Agarose gel is incompletely solubilized.	Check that incubation temperature is 50-60°C. Vortex the gel mixture every 2-3 minutes during the incubation. Use higher volume of GSS if necessary (see above).
	The pH of the electrophoresis buffer is too high, resulting in inefficient DNA binding.	Do not re-use the electrophoresis buffer.
	Wash Solution did not contain ethanol.	Check that ethanol was added to the concentrated WS, and that the cap on the bottle was tightly sealed.
	The wrong volume of Elution Solution was used.	Use 30-50 µl of Elution Solution or water. Check that the Elution Solution completely covers the membrane.
Poor performance in downstream applications	Too much salt in the eluate.	Elute the DNA in water or 10 mM Tris-HCl, pH 8.0.
	Residual ethanol from the Wash Solution remained on the column and was eluted with the DNA.	Recentrifuge the column for 2 minutes after the wash step (step 5) to remove residual Wash Solution.
	Eluate is contaminated with agarose gel.	The gel slice is incompletely solubilized. Repeat the procedure.

Reference

Vogelstein, B., and Gillespie, D., Proc. Natl. Acad. Sci. USA, **76**, 615 (1979)

Related Products	Product No.	Related Products	Product No.
Ethanol, absolute, Molecular Biology Grade	E7023	TBE buffer, 5X concentrate	T6400
		TAE buffer, 10X concentrate	T9650
Agarose, routine use	A9539	*Taq* DNA Polymerase	D1806,
Agarose, low melting point	A9414		D4545
Agarose, high resolution	A4718	Deoxynucleotide (dNTP) mix	D7295
Ethidium Bromide solution, 10 mg/ml	E1510	AutoPAGE™ 4.0% acrylamide	P0468
Gel Loading Solution	G2526	halfBD dye terminator sequencing reagent	H1407
Lambda DNA EcoR I Hind III digest	D9281	TBE buffer, 10X for sequencing	T4415

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