GenElute™ Gel Extraction Kit

Product Code **NA 1010**

ProductInformation

TECHNICAL BULLETIN

Product Description

The GenElute™ Gel Extraction Kit is designed for the rapid purification of DNA of 50 bp to 10 kb in length, from standard or low-melting agarose gels in TAE or TBE buffer. A typical recovery is 50 to 55% with recoveries as high as 80%.

The GenElute Gel Extraction Kit combines silica-binding 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 guanidine thiocyanate, which can dissolve an agarose gel slice from gels run in either TBE or TAE buffer. It also contains a pH indicator which allows the gel slice to be visualized easily as well as indicating whether the pH is optimal for binding. 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 Code**	**Quantity**
Gel Solubilization Solution	S 4064	140 ml
Wash Solution Concentrate	W 3637	12 ml
Elution Solution (10 mM Tris-HCl, pH 9.0)	E 9027	5 ml
GenElute™ Miniprep Binding Columns in Tubes	G 6415	70 each
Collection Tubes, 2 ml	T 7813	70 each

Storage

Store the kit at room temperature.

Equipment and Reagents Required But Not Provided

- Cutting tools for gel (scalpel handle and blades, S 2896 and S 2646, respectively) or razor blades
- Pipettors and tips
- Water bath or heating block at 50-60 °C
- Ethanol (95-100%), E 7148 or E 7023
- Isopropanol (99+%), I 9516 or I 0398
- Microcentrifuge and tubes
- Water, Molecular Biology Reagent, W 4502
- 3 M Sodium Acetate Buffer, pH 5.2, S 7899

Precautions and Disclaimer

The GenElute Gel Extraction Kit is for laboratory use only. Not for drug, household or other uses. The Gel Solubilization Solution contains guanidine thiocyanate, 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 (MSDS).

Preparation Instructions

1. **Wash Solution:** Dilute the entire 12 ml of the Wash Solution Concentrate with 48 ml of 95-100% ethanol prior to initial use. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of ethanol.

2. **Gel Solubilization Solution:** Guanidine thiocyanate will precipitate out of solution if stored at temperatures less than 20-25 °C. Check to ensure that this solution is completely dissolved and that no crystals have precipitated out of solution. If crystals are present, incubate at 37-50 °C with periodic mixing for 5 minutes or until the crystals dissolve.

3. **Agarose Gel Electrophoresis:** TAE buffer is recommended over TBE buffer for use as the running buffer for DNA agarose gels. The borate present in TBE reacts with agarose to form a noncovalent tetrahydroborate complex, which reduces the recovery of DNA, regardless of the system used to purify the DNA. This interaction is avoided by using TAE buffer.

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

Procedure

All centrifugations (spins) are performed at 12,000 to 16,000 x g (See Appendix I to convert g-force to RPM).

1. **Excise band.** Excise the DNA fragment of interest from the agarose gel with a clean, sharp scalpel or razor blade. Trim away excess gel to minimize the amount of agarose.

2. **Weigh gel.** Weigh the gel slice in a colorless 1.5 to 2.0 ml microcentrifuge tube.

3. **Solubilize gel.** Add 3 gel volumes of the Gel Solubilization Solution to the gel slice. 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 slice is completely dissolved. Vortex briefly every 2-3 minutes during incubation to help the gel dissolve.

   Note: To dissolve a >2% agarose gel adequately, it is necessary to increase the ratio of the Gel Solubilization Solution to the gel weight to 6:1.

4. **Check the color of the mixture.** Once the gel slice is completely dissolved make sure that the color of the mixture is yellow (similar to fresh Gel Solubilization Solution with no gel slice). If the color of the mixture is purple/brown, add 10 µl of 3 M sodium acetate buffer, pH 5.2, and mix. The color should now be yellow. If not, add another 10 µl of 3 M sodium acetate buffer, pH 5.2, and mix. Continue this process until the mixture is yellow.

5. **Add isopropanol.** Add 1 gel volume of 99+% isopropanol and mix briefly until homogenous.

6. **Bind DNA.** Load the entire DNA sample onto the GenElute Miniprep binding column that is assembled in a 2 ml collection tube. The binding column for this kit has a blue o-ring (not to be confused with other GenElute kits). If the volume of the gel mixture is >700 µl, load the sample onto the column in two portions. Centrifuge for 1 minute after loading the column each time. Discard the flow-through liquid.

7. **Optional: Remove trace amounts of agarose.** Add 500 µl of Gel Solubilization Solution to the binding column and incubate for 1 minute at room temperature. Centrifuge for 1 minute. Remove the binding column from the collection tube and discard the flow-through liquid. Place the binding column back into the collection tube.

   Note: This step is only necessary for very sensitive downstream applications such as direct sequencing, microinjection, and *in vitro* transcription.

8. **Wash column.** Add 600 µl of the diluted Wash Solution to the binding column. If the DNA is to be used for downstream processes that are salt sensitive (i.e. blunt end ligations, direct sequencing, etc.), incubate the column for 2-5 minutes after the addition of Wash Solution to ensure the removal of trace amounts of salt. Otherwise, incubation is not required. Centrifuge for 1 minute. Remove the binding column from the collection tube and discard the flow-through liquid. Place the binding column back into the collection tube and centrifuge again for 2 minutes without any additional wash solution to remove excess ethanol.

9. **Elute DNA.** Transfer the binding column to a fresh collection tube. Add 50 µl of Elution Solution or nuclease-free water to the center of the membrane and incubate for 1 minute. Centrifuge for 1 minute.

   Note: To increase DNA concentration, a minimum of 30 µl of the Elution Solution or nuclease-free water may be used to elute the DNA from the column. The Elution Solution must be placed directly onto the membrane, not the side of the column, to completely elute bound DNA. If using water to elute, make sure that the pH is $\geq$ 5.0.

Troubleshooting Guide

Problem	Reason	Solution
Poor or low recovery	Ratio of Gel Solubilization Solution to gel is incorrect.	Use a ratio of 3:1, volume (µl) of Gel Solubilization Solution to weight (mg) of gel fragment. For agarose gels >2%, use a ratio of 6:1.
	Agarose gel is incompletely solubilized.	Check that the incubation temperature is 50-60 °C. Vortex the gel mixture every 2-3 minutes during the incubation. Use a higher volume of Gel Solubilization Solution if necessary (see above) and incubate longer.
	The pH of the electrophoresis buffer is too high, resulting in inefficient DNA binding.	Use fresh electrophoresis buffer. Check the color of the Gel Solubilization Solution to make sure that it is yellow. Add 10 µl of 3M sodium acetate buffer, pH 5.2, if it is purple to brown.
	Wash Solution did not contain ethanol.	Check that the ethanol was added to the Wash Solution Concentrate and that the cap on the bottle was tightly sealed.
	The wrong volume of Elution Solution was used.	Use 30-50 µl of the Elution Solution or water. Check that the Elution Solution completely covers the membrane.
	Elution Solution was not added directly to the membrane.	Add the Elution Solution directly to the column membrane, not the side of the column. Do not touch or pierce the membrane.
	A gelatinous precipitate formed upon the addition of isopropanol.	This is probably due to agarose interacting with the borate in TBE to form tetrahydroborate complexes. Alternatively, the agarose was not completely dissolved prior to adding the isopropanol. Mix the solution until homogenous. The precipitate should dissolve. If not, incubate the gel mixture at 50-60 °C for 5 minutes, or until the precipitate is completely dissolved. Perform the optional Gel Solubilization Solution wash to remove trace amounts of agarose.
Poor performance in downstream applications	The eluate contains too much salt.	Incubate the column for 5 minutes after adding 600 µl of Wash Solution, then spin through.
	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 8) to remove residual Wash Solution.
	Eluate is contaminated with agarose gel.	The gel slice was incompletely solubilized prior to loading the column. Perform the optional Gel Solubilization Solution wash to remove trace amounts of agarose.

Reference

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

Related Products	Product Code	Related Products	Product Code
Agarose, low melting point	A 9414	TAE buffer, 10X concentrate	T 9650
Agarose, high resolution	A 4718	JumpStart™ Red*Taq*™ ReadyMix™	P 0982
Ethidium Bromide solution, 10 mg/ml	E 1510	Deoxynucleotide (dNTP) mix	D 7295
Gel Loading Solution	G 2526	AutoPAGE™ 4.0% acrylamide	P 0468
Lambda DNA *Eco*R I *Hin*d III digest	D 9281	halfBD Dye Terminator Sequencing Reagent	H 1407
GenElute™ Minus EtBr Spin Columns	5-6501	TBE buffer, 10X for sequencing	T 4415
TBE buffer, 5X concentrate	T 6400	GenElute™ Agarose Spin Columns	5-6500

Appendix I

Note: All centrifugation speeds are given in units of g. Please refer to Table 1 for information on converting g-force to rpm. *If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time proportionally. Spin until all liquid passes through the column.*

Table 1. Conversion of Centrifugal Force (in units of *g*) to RPM for Common Rotors

Centrifuge	Rotor	Tubes (max)	Radius (cm)	RPM at 12,000 x *g*	RPM at 16,000 x *g*
EPPENDORF					
5410		12	5.8	13,555	15,652
5415C	F45-18-11	18	7.3	12,124	14,000
5415D&R	F45-24-11	24	8.3	11,392	13,155
5417C,D,&R	F45-30-11	30	9.5	10,634	12,279

See table above for spin speeds in rpm for selected common centrifuges and rotors. The correct rpm for unlisted rotors can be calculated using the formula:

$$RPM = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*; *r* = radius of the rotor in cm; *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force

‡The PCR process is covered by patents owned by Hoffman-LaRoche, Inc. SM 7/01