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ProductInformation

GenElute™ Gel Extraction Kit

Product Code **NA1111**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The GenElute™ Gel Extraction Kit is designed for the rapid purification of 50 bp to 10 Kb linear DNA fragments and plasmids from standard or low-melting agarose gels. This kit can also be used to purify DNA from polyacrylamide gels.

The GenElute Gel Extraction Kit combines silica-binding technology with the convenience of a spin or vacuum column format. DNA fragments of interest are extracted from slices of an agarose gel by solubilizing the gel. The Gel Solubilization Solution can dissolve an agarose slice from gels run in either TBE or TAE buffer. This solution also contains a pH indicator that allows the gel slice to be visualized easily and indicates whether the pH is optimal for DNA binding. The extracted DNA fragments are then selectively adsorbed onto a silica membrane in the presence of the Gel Solubilization Solution. Contaminants are removed by a simple spin or vacuum 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.

A typical recovery is 50-55% with recoveries as high as 80%. Each column can bind up to 10 µg of DNA, and up to 3.5 g of agarose can be processed per column.

Precautions and Disclaimer

The GenElute Gel Extraction Kit is for laboratory use only and is not intended for drug, household, or other uses. The Gel Solubilization Solution contains a chaotropic salt, which is an irritant. The Column Preparation Solution is also an irritant. Wear gloves, safety glasses, and suitable protective clothing when handling this solution or any reagents provided with the kit. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Components

Sufficient for 70 preparations

Reagent Provided	Product Code	Quantity
Column Preparation Solution	C 2112	60 ml
Gel Solubilization Solution	G 8668	140 ml
Wash Solution Concentrate G	W 2139	12 ml
Elution Solution (10 mM Tris-HCl, pH 9.0)	E 9027	6 ml
GenElute Binding Column G	C 6863	70 each
Collection Tubes, 2 ml	T 7813	2 X 70 each

Equipment and Reagents Required But Not Provided

(Product Codes have been given where appropriate)

- Cutting tools for gel (scalpel 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, 99-100% (I 9516 or I 0398)
- Microcentrifuge and tubes
- Water, Molecular Biology Reagent (W 4502)
- 3 M Sodium Acetate Buffer, pH 5.2 (S 7899)

Storage

Store the kit at room temperature

Preparation Instructions

1. **Wash Solution:** Dilute the entire 12 ml of the Wash Solution Concentrate G 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:** The Gel Solubilization Solution will precipitate out of solution if stored at temperatures less than 18-25 °C. Check to ensure that this solution is completely dissolved and that no crystals are present. If crystals are present, incubate at 37-50 °C with periodic mixing until the crystals dissolve (approximately 5 minutes).

3. **Agarose Gel Electrophoresis:** Use fresh electrophoresis running buffer. Electrophoresis buffer, which has been used repeatedly, will reduce the DNA recovery efficiency. Minimize examination of ethidium bromide-stained gels with an UV transilluminator. If possible, use a transilluminator equipped with a long-wavelength (302 nm) UV light source, as this will minimize the damaging effects of UV light on nucleic acids.

4. **Elution Solution:** If purifying plasmid DNA or large linear DNA fragments (>3 Kb) preheat the Elution Solution to 65 °C prior to use.

Procedure

A. Spin Procedure for Agarose Gels

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

1a. **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.

2a. **Weigh gel.** Weigh the gel slice in a tared colorless tube.

3a. **Solubilize gel.** Add 3 gel volumes of the Gel Solubilization Solution to the gel slice. In other words, for every 100 mg of agarose gel, add 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 dissolve the gel.

Note: To adequately dissolve a gel with an agarose concentration greater than 2%, it is necessary to increase the ratio of the Gel Solubilization Solution volume to the gel weight to 6:1.

4a. **Prepare binding column.** Preparation of the binding column can be completed while the agarose is being solubilized in step 3a. Place the GenElute Binding Column G into one of the provided 2 ml collection tubes. Add 500 µl of the Column Preparation Solution to each binding column. Centrifuge for 1 minute. Discard flow-through liquid.

Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.

5a. **Check the color of the mixture.** Once the gel slice is completely dissolved (step 3a) make sure the color of the mixture is yellow (similar to fresh Gel Solubilization Solution with no gel slice) **prior** to proceeding to the following step. If the color of the mixture is red, add 10 µl of the 3 M Sodium Acetate Buffer, pH 5.2, and mix. The color should now be yellow. If not, add the 3 M Sodium Acetate Buffer, pH 5.2, in 10 µl increments until the mixture is yellow.

6a. **Add isopropanol.** Add 1 gel volume of 100% isopropanol and mix until homogenous. For a gel with an agarose concentration greater than 2%, use 2 gel volumes of 100% isopropanol.

7a. **Bind DNA.** Load the solubilized gel solution mixture from step 6a into the binding column that is assembled in a 2 ml collection tube. It is normal to see an occasional color change from yellow to red once the sample is applied to the binding column. If the volume of the gel mixture is >700 µl, load the sample onto the column in 700 µl portions. Centrifuge for 1 minute after loading the column each time. Discard the flow-through liquid.

Note: Do not be alarmed if the flow-through has changed color.

8a. **Wash column.** Add 700 µl of Wash Solution (diluted from Wash Solution Concentrate G as described under Preparation Instructions) to the binding column. 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 1 minute without any additional wash solution to remove excess ethanol. Residual Wash Solution will not be completely removed unless the flow-through is discarded before the final centrifugation.

9a. **Elute DNA.** Transfer the binding column to a fresh collection tube. Add 50 µl of Elution Solution to the center of the membrane and incubate for 1 minute. Centrifuge for 1 minute. For efficient recovery of intact plasmid DNA, preheat the elution solution to 65 °C prior to adding it to the membrane. Eluting at 65 °C improves plasmid DNA recoveries by 2 to 3-fold. Yields of large linear DNA fragments (>3 Kb) can also be increased by up to 20% by preheating the elution solution to 65 °C.

Note: To increase the concentration of the eluted DNA, the volume of Elution Solution may be reduced to 25 µl. Yields are approximately 25% lower when eluting with 25 µl as opposed to 50 µl.

B. Vacuum Procedure for Agarose Gels

The GenElute Miniprep Binding Columns are designed for use with any vacuum manifold with luer connectors.

1b. **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.

2b. **Weigh gel.** Weigh the gel slice in a tared colorless tube.

3b. **Solubilize gel.** Add 3 gel volumes of the Gel Solubilization Solution to the gel slice. In other words, for every 100 mg of agarose gel, add 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 dissolve the gel.

Note: To adequately dissolve a gel with an agarose concentration greater than 2%, it is necessary to increase the ratio of the Gel Solubilization Solution volume to the gel weight to 6:1.

4b. **Prepare binding column.** Preparation of the binding column can be completed while the agarose is being solubilized in step 3b. Place the GenElute Binding Column G onto the vacuum manifold. Apply vacuum and add 500 µl of the Column Preparation solution to the column. Allow the Column Preparation Solution to pass completely though the column.

Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.

5b. **Check the color of the mixture.** Once the gel slice is completely dissolved (step 3b) make sure the color of the mixture is yellow (similar to fresh Gel Solubilization Solution with no gel slice) **prior** to proceeding to the following step. If the color of the mixture is red, add 10 µl of 3 M Sodium Acetate, pH 5.2, and mix. The color should now be yellow. If not, add the 3 M Sodium Acetate Buffer, pH 5.2, in 10 µl increments until the mixture is yellow.

6b. **Add isopropanol.** Add 1 gel volume of 100% isopropanol and mix briefly until homogenous. For a gel with an agarose concentration greater than 2%, use 2 gel volumes of 100% isopropanol.

7b. **Bind DNA.** With vacuum applied, load the solubilized gel solution mixture from step 6b into the binding column that is attached to the vacuum manifold. Be careful not to overfill. Allow the mixture to pass through the column. It is normal to see an occasional color change from yellow to red once the sample is applied to the column. Do not be alarmed if the flow-through has changed color.

8b. **Wash column.** Add 700 µl of Wash Solution (diluted from Wash Solution Concentrate G as described under Preparation Instructions) to the binding column and allow it to pass through.

9b. **Transfer column.** Remove the binding column from the vacuum manifold and transfer it to a clean 2 ml collection tube. Centrifuge for 1 minute at 12,000 to 16,000 x *g* to remove excess ethanol.

10b. **Elute DNA.** Transfer the binding column to a fresh collection tube. Add 50 µl of Elution Solution to the center of the membrane and incubate for 1 minute. Centrifuge for 1 minute. For efficient recovery of intact plasmid DNA, preheat the elution solution to 65 °C prior to adding it to the membrane. Eluting at 65 °C improves plasmid DNA recoveries by 2 to 3-fold. Yields of large linear DNA fragments (>3 Kb) can also be increased by up to 20% by preheating the elution solution to 65 °C.

Note: To increase the concentration of the eluted DNA, the volume of Elution Solution may be reduced to 25 µl. Yields are approximately 25% lower when eluting with 25 µl as opposed to 50 µl.

C. Extraction of DNA from Polyacrylamide Gels
The GenElute Gel Extraction Kit is designed for isolating DNA from agarose gels; however, the kit can also be used to isolate DNA from polyacrylamide gels when using the following "crush and soak" method. The Gel Diffusion Buffer, which is not included in the kit, must be prepared prior to beginning the procedure. All centrifugations (spins) are performed at 12,000 to 16,000 x *g* (See Appendix I to convert *g*-force to rpm).

1c. **Prepare Gel Diffusion Buffer.** The Gel Diffusion Buffer consists of 0.1% SDS, 1 mM EDTA, 10 mM magnesium acetate, and 500 mM ammonium acetate, pH 8.0. To make 100 ml, add 50 ml nuclease-free water to an appropriately sized beaker and stir. Add the components in the order that they are listed (see Table 1) while stirring. Continue to mix until all components are thoroughly dissolved. Bring up to 100 ml with nuclease-free water. The ammonium acetate must be added last to avoid precipitation of the SDS.

Table 1.

Component	Product Code	Amount for 100 ml
Water, Molecular Biology grade	W 4502	To 100 ml
10% SDS Solution	L 4522	1 ml
0.5 M EDTA, pH 8.0	E 7889	0.2 ml
Magnesium acetate	M 5661	0.215 g
Ammonium acetate	A 7262	3.854 g

Note: If the SDS precipitates, incubate at 37-50 °C with periodic mixing until the crystals dissolve (approximately 5 minutes).

2c. **Excise Band.** Excise the DNA fragment of interest from the polyacrylamide gel with a clean, sharp scalpel or razor blade. Trim away excess gel to minimize the amount of polyacrylamide. Transfer the gel slice into a tared 1.5 ml colorless microcentrifuge tube.

3c. **Crush Gel.** Crush the gel slice using a clean disposable pipette tip (i.e., a 200 µl pipette tip) or similar device by compressing it between the side of the tube using the pipette tip. The smaller the pieces the better. Breaking the gel slice into tiny pieces results in up to 50% greater yields of DNA due to the increased surface area available for passive diffusion.

Note: If pieces of gel become lodged in the pipette tip, they can easily be removed by briefly centrifuging the pipette tip in the opened microcentrifuge tube. The tip can then be discarded.

4c. **Weigh gel.** Weigh the gel in the tube in which it is crushed.

5c. **Diffuse DNA from gel.** Resuspend the gel pieces in 2 gel volumes of Gel Diffusion Buffer. In other words, for every 50 mg of gel, add 100 µl of Gel Diffusion Buffer. Make certain that all pieces are submerged in the Gel Diffusion Buffer. Incubate at 50 °C for a minimum of 30 minutes. For the best yields, the incubation time can be extended overnight.

6c. **Remove Residual Polyacrylamide.** Spin the crushed gel/buffer mixture at 12,000 to 16,000 x *g* for 1 minute and carefully remove the supernatant to a new microcentrifuge tube being careful to avoid transferring fragments of polyacrylamide. Alternatively, residual polyacrylamide can be removed by passing the mixture through a disposable plastic column (Product Code 5-6500).

7c. **Optional**: For optimal yield, add an additional 0.5 gel volume of Gel Diffusion Buffer to the pelleted polyacrylamide. Vortex briefly to resuspend the pellet and repeat steps 5c and 6c. Pool the supernatant and proceed to the next step.

8c. Measure the volume of recovered supernatant and add 3 volumes of the Gel Solubilization Solution. For every 100 µl of supernatant, add 300 µl of Gel Solubilization Solution.

9c. Continue with step 4 of either the Vacuum or Spin Procedure.

Troubleshooting Guide

Problem	Reason	Solution
Low recovery	Ratio of Gel Solubilization Solution to gel is incorrect.	The ratio of the volume (µl) of Gel Solubilization Solution to the weight (mg) of gel fragment should be 3:1. For gels with agarose concentrations greater than 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 larger 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. If not, add the 3 M Sodium Acetate Buffer, pH 5.2, in 10 µl increments until the mixture is yellow.
	Wash Solution did not contain ethanol.	Check that ethanol was added to the Wash Solution Concentrate G and that the cap on the bottle was tightly sealed.
	The wrong volume of Elution Solution was used.	Use 25-50 µl of the Elution Solution. Check that the Elution Solution completely covers the membrane.
	Isopropanol was not added in step 6.	Isopropanol facilitates DNA binding to the GenElute Binding Columns G. Add one gel volume of isopropanol to the DNA mixture prior to binding. For gels with agarose concentrations greater than 2%, use two gel volumes.
	The GenElute Binding Columns G were not washed with Column Preparation Solution.	Wash the binding columns with 500 µl of Column Preparation Solution prior to loading DNA onto the columns.
	Did not preheat elution solution to 65 °C prior to eluting.	Eluting at 65 °C improves plasmid DNA recoveries by 2 to 3-fold. Yields of large linear fragments (>3 Kb) can be increased by up to 20% by preheating the Elution Solution to 65 °C.
	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 the 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.
Poor performance in downstream applications	The eluated DNA contains too much salt.	Incubate the column for 5 minutes after adding 700 µl of Wash Solution, then spin/vacuum through.
	Residual ethanol from the Wash Solution remained on the column and was eluted with the DNA.	Recentrifuge the column for 1 minute after the wash step to remove residual Wash Solution.
	Eluated DNA is contaminated with agarose gel.	The gel slice is incompletely solubilized. Vortex the gel mixture every 2-3 minutes during the incubation. Use a larger volume of Gel Solubilization Solution if necessary (see above) and incubate longer.

Appendix I

Note: All centrifugation speeds are given in units of *g*. Please refer to Table 2 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 2.
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

References

1. Vogelstein, B., and Gillespie, D., *Proc. Natl. Acad. Sci. USA*, **76**, 615 (1979)
2. Sambrook, J., and Russell, D.W., Molecular Cloning: A Laboratory Manual, 3rd ed., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001) pp. 5.51-5.54

Related Products	Product Code	Related Products	Product Code
Agarose, low melting point	A 9414	TAE buffer, 10x concentrate	T 9650
Agarose, for routine use	A 9539	JumpStart™ Red*Taq*™ ReadyMix™	P 0982
Water, Molecular Biology Grade	W 4502	10% SDS solution	L 4522
Ammonium acetate	A 7262	0.5 M EDTA, pH 8.0	E 7889
Magnesium acetate	M 5661	GenElute Agarose Spin Columns	5-6500
Ethidium Bromide solution, 10 mg/ml	E 1510	GenElute Minus EtBr Spin Columns	5-6501
Gel Loading Solution	G 2526	Deoxynucleotide (dNTP) mix	D 7295
Lambda DNA *Eco*R I, *Hin*d III digest	D 9281	AutoPAGE™ 4.0% acrylamide	P 0468
TBE buffer, 10x for sequencing	T 4415	halfBD Dye Terminator Sequencing Reagent	H 1407
TBE buffer, 5x concentrate	T 6400		

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

SM/MAM 7/03