Gel Extraction Kits

NucleoGen
BIOTECHNOLOGY

Contents

Kit Contents

Catalog No.	5215	5212
Number of preparations	50	200
Nucleogen Spin Columns	50	200
Collection tubes (2 ml)	50	200
Gel Extraction Buffer *	+	+
Wash C Solution (concentrate)	+	+
Elution Buffer	+	+

* Gel Extraction Buffer contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling.

Principle

The Gel Extraction Buffer is optimized for efficient recovery of DNA and removal of contaminants. DNA adsorbs to the silica-membrane in the presence of high salt while contaminants pass through the column. Impurities are efficiently washed away, and the pure DNA is eluted with Elution Buffer or water. The Gel Extraction spin columns offer two handling options — as an alternative to processing the spin columns in a microcentrifuge, they can now also be used on any commercial vacuum manifold with luer connectors.

Adsorption to Gel Extraction membrane — salt & pH dependence

The Gel Extraction membrane is uniquely adapted to isolate DNA from both aqueous solutions and agarose gels, and up to 10 ug DNA can bind to Gel Extraction column.
The Gel Extraction Buffers in Gel Extraction Kits provide the correct salt concentration and pH for adsorption of DNA to the Gel Extraction membrane. The adsorption of nucleic acids to silica-gel surfaces occurs only in the presence of a high concentration of chaotropic salts, which modify the structure of water.

Adsorption of DNA to Gel Extraction membrane also depends on pH. Adsorption is typically 95% if the pH is ≤7.5, and is reduced drastically at higher pH. If the loading mixture pH is >7.5, the optimal pH for DNA binding can be obtained by adding a small volume of 3 M sodium acetate, pH 5.0.

pH indicator in solubilization and Gel Extraction Buffer

The binding and solubilization Gel Extraction Buffer has been specially optimized for use with the Gel Extraction membrane. Gel Extraction Buffer contains a pH indicator, allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH ≤7.5, and the pH indicator in Gel Extraction Buffer appears yellow in this range. If the pH is >7.5, which can occur if the agarose gel electrophoresis buffer is frequently used or incorrectly prepared, the binding mixture turns orange or violet. This means that the pH of the sample exceeds the buffering capacity of Gel Extraction Buffer and DNA adsorption will be inefficient. In this case, the pH of the binding mixture can easily be corrected by addition of a small volume of 3 M sodium acetate, pH 5.0, before proceeding with the protocol.

Gel Extraction Buffer does not contain NaI. Residual NaI may be difficult to remove from DNA samples, and reduces the efficiency of subsequent enzymatic reactions such as blunt-end ligation.

Washing

During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, ethidium bromide, oils, and detergents (e.g., DMSO, Tween® 20) do not bind to the membrane, but flow through the column. Salts are quantitatively washed away by the ethanol-containing Wash C Solution. Any residual Wash C Solution, which may interfere with subsequent enzymatic reactions, is removed by an additional centrifugation step.

Elution in low-salt solutions

Elution efficiency is strongly dependent on the salt concentration and pH of the Elution Buffer. Contrary to adsorption, elution is most efficient under basic conditions and low salt concentrations. DNA is eluted with 50 or 30 ul of the provided Elution Buffer (10 mM Tris-Cl, pH 8.5), or Water. The maximum elution efficiency is achieved between pH 7.0 and 8.5.
When using water to elute, make sure that the pH is within this range. In addition, DNA must be stored at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent. Elution with TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) is possible, but not recommended because EDTA may inhibit subsequent enzymatic reactions.

DNA yield and concentration

DNA yield depends on the following three factors: the volume of Elution Buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column.
100–200 ul of elution buffer completely covers the Gel Extraction membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with ≤50 ul requires the buffer to be added directly to the center of the membrane, and if elution is done with the minimum recommended volume of 30 ul, an additional 1 min incubation is required for optimal yield. DNA will be up to 1.7 times more concentrated if the Nucleogen Gel Extraction column is incubated for 1 min with 30 ul of elution buffer, than if it is eluted in 50 µl without incubation.

Important Notes

Please read the following notes before starting any of the Nucleogen Gel Extraction procedures.

Before equipment

- The yellow color of Gel Extraction Buffer indicates a pH ≤7.5.
- **Add ethanol (96-100%) to Wash C Solution** before use (see bottle label for volume).
- Isopropanol (100%) and a heating block or water bath ot 50°C are required.
- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a Conventional table-top microcentrifuge.
- 3 M sodium acetate, pH 5.0, may be necessary.

Centrifugation notes:

- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional, table-top microcentrifuge.

Vacuum notes:

- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.
- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere

Gel Extraction Kit Protocol
Using a Microcentrifuge

Please read "Important Notes" on pages 4 before starting.

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or Low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA cleanup from enzymatic reactions.
For DNA cleanup from enzymatic reactions using this protocol, add 3 volumes of Gel Extraction Buffer and 1 volume of isopropanol to the reaction, mix, and proceed with step 6 of the Protocol.

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.

2. **Weigh the gel slice in a colorless tube.**
   Add 3 volumes of Gel Extraction Buffer to 1 volume of gel (100 mg ~ 100 ul).
   For example, add 300 ul Gel Extraction Buffer to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Gel Extraction Buffer. The maximum amount of gel slice per spin column is 400 mg; for gel slices >400 mg use more than one spin column.

3. **Incubate at 50°C for 10 min (or until the gel slice has completely dissolved).**
   To help dissolve gel, mix by vortexing the tube every 2-3 min during the incubation.
   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

4. **After the gel slice has dissolved completely, check that the color of the mixture is Yellow (similar to Gel Extraction Buffer).**
   If the color of the mixture is orange or violet, add 10 ul 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
   The adsorption of DNA to the membrane is efficient only at pH ≤7.5.
   Gel Extraction Buffer contains a pH indicator which is yellow at pH ≤7.5 and orange or violet at Higher pH, allowing easy determination of the optimal pH for DNA binding.

5. **Add 1 gel volume of isopropanol to the sample and mix. Transfer the mixture to a spin column.**
   For example, if the agarose gel slice is 100 mg, add 100 ul isopropanol. This step Increases the yield of DNA fragments <500 bp and >4kb. For DNA fragments Between 500 bp and 4 kb, addition of isopropanol has no effect on yield.
   Do not centrifuge the sample at this stage.

6. **To bind DNA, apply the sample to the spin column, and centrifuge for 1 min.**
   Remove the spin column from the tube and discard flow-through from same collection tube.
   Reinsert the spin column in the same collection tube.

7. **(Optional): Add 500 ul Gel Extraction Buffer to spin column and centrifuge for 1 min.**
   This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.

8. **To wash, add 750 ul Wash C Solution to spin column and centrifuge for 1 min**.
   Note: If the DNA will be used for salt sensitive application, such as blunt-end ligation and direct sequencing, let the column stand 2-5 min after addition of Wash C Solution, before centrifuging.

9. **Discard the flow-through and centrifuge the spin column for an additional 1 min at 13,000 rpm (~17,900 x g). Place spin column into a clean 1.5 ml microcentrifuge tube.**
   IMPORTANT: Residual ethanol from Wash C Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation.

10. **To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the membrane and centrifuge the column for 1min. Altematively, for increased DNA concentration, add 30 ul Elution Buffer to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.**

Gel Extraction Kit Protocol
Using a Vacuum Manifold

Please read "Important Notes" on pages 4 before starting.

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.

2. **Weigh the gel slice in a colorless tube.**
   Add 3 volumes of Gel Extraction Buffer to 1 volume of gel (100 mg ~ 100 µl).
   For example, add 300 ul of Gel Extraction Buffer to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Gel Extraction Buffer. The maximum amount of gel slice per spin column is 400 mg; for gel slices >400 mg use more than one spin column.

3. **Incubate at 50°C for 10 min (or until the gel slice has completely dissolved).**
   To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.
   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.
   - Insert each spin column into a luer connector on the Luer Adapter(s) in the manifold.

4. **After the gel slice has dissolved completely, check that the color of mixture is yellow (similar to Gel Extraction Buffer).**
   If the color of the sample is orange or violet, add 10 ul of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
   The adsorption of DNA to the membrane is efficient only at pH ≤7.5.
   Gel Extraction Buffer contains a pH indicator which is yellow at pH ≤7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. **Add 1 gel volume of isopropanol to the sample and mix.**
   For example, if the agarose gel slice is 100 mg, add 100 µl isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.

6. **To bind DNA, pipet the sample onto the spin column and apply vacuum. After the sample has passed through the column, switch off vacuum source.**
   The maximum volume of the column reservoir is 800 ul. For sample volumes of more than 800 ul, simply load again.

7. **(Optional): Add 500 ul of Gel Extraction Buffer to spin column and apply vacuum.**
   This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.

8. **To wash, add 750 ul of Wash C Solution to spin column and apply vacuum.**
   Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Wash C solution before applying vacuum.

9. **Transfer spin column to a clean 1.5 ml microcentrifuge tube or to a provided 2 ml collection tube. Centrifuge for 1 min at 13,000 rpm (~17,900 x g).**
   IMPORTANT: This spin is necessary to remove residual ethanol (Wash C solution).

10. **Place spin column in a clean 1.5 ml microcentrifuge tube.**

11. **To elute DNA, add 50 ul of Elution Buffer (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the membrane and centrifuge the column for 1 min at 13,000 rpm (~17,000 x g). Alternatively, for increased DNA concentration, add 30 ul elution buffer, let stand for 1 min, and then centrifuge for 1 min.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise.

	Comments and suggestions
Low or no yield	
a) Wash C Solution did not Contain ethanol	Ethanol must be added to Wash C Solution (concentrate) before use. Repeat procedure with correctly prepared Wash C Solution.
b) Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low-salt buffer (e.g., Elution Buffer: 10 mM Tris·Cl, pH 8.5) or water.
c) Elution Buffer Incorrectly dispensed	Add Elution Buffer to the center of the membrane to ensure that the buffer completely covers the membrane. This is particularly important when using small elution volumes (30 ul).
d) Gel slice incompletely solubilized	After addition of Gel Extraction Buffer to the gel slice, mix by vortexing the tube every 2-3 minutes during the 50°C incubation. DNA will remain in any undissolved agarose.
e) pH of electrophoresis buffer too high (binding mixture turns orange or violet)	The electrophoresis buffer has been repeatedly used or incorrectly prepared, resulting in a sample pH that exceeds the buffering capacity of Gel Extraction Buffer and leads to inefficient DNA binding. Add 10 ul of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture will turn yellow indicating the correct pH for DNA binding. Even for binding mixtures with only small color changes (slight orange color), add the 10 ul sodium acetate.
f) Gel slice was too large (>400 mg)	70–80% recovery can only be obtained from ≤400 mg gel slice per spin column. For gel slices >400 mg, use multiple spin columns.
g) Cloudy and gelatinous appearance of sample mixture after addition of isopropanol	This may be due to salt precipitation, and will disappear upon mixing the sample. Alternatively, the gel slice may not be completely solubilized. In this case, apply the mixture to the spin column, centrifuge, and then add 500 ul Gel Extraction Buffer to the column. Let stand for 1 min at room temperature, and then centrifuge and continue with the procedure. This additional wash will solubilize remaining agarose.
DNA does not perform well, e.g., in ligation reactions	
a) Salt concentration in eluate too high	Modify the wash step by incubating the column for 5 min at room temperature after adding 750 ul of Wash C Solution, then centrifuge.
b) Elution contains residual ethanol	Ensure that the wash flow-through is drained from the collection tube and that the spin column is then centrifuged at 13,000 rpm (~17,900 x g) for an additional 1 min.
c) Elution contaminated with agarose	The gel slice is incompletely solubilized or weighs >400 mg. Repeat procedure, including the optional Gel Extraction Buffer column-wash step.
d) Eluate contains denatured ssDNA, which appears as smaller smeared band on an analytical gel	Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95°C for 2 min, and allow the tube to cool slowly to room temperature. Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 10 mM NaCl. The salt and buffering agent promote the renaturation of DNA strands. However the salt concentration of the eluate must then be considered for subsequent applications.

Additional Protocol

Protocol for DNA fragments extract from polyacrylamide gels

Please read "Important Notes" on pages 4 before starting.

1. **Excise the gel slice containing the DNA band with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing excess polyacrylamide.
2. **Weigh the gel slice. Add 1–2 volumes of Diffusion Buffer to 1 volume of gel (i.e., 100–200 ul for each 100 mg of gel).**
   - Diffusion Buffer: 0.5 M ammonium acetate; 10 mM magnesium acetate; 1 mM EDTA, pH 8.0; 0.1% SDS.
3. **Incubate at 50°C for 30 min.**
4. **Centrifuge the sample for 1 min.**
5. **Carefully remove the supernatant using a pipet or a drawn-out Pasteur pipet. Pass the supernatant through a disposable plastic column or a syringe containing either a Whatman GF/C filter or packed, siliconized glass wool to remove any residual polyacrylamide.**
6. **Determine the volume of the recovered supernatant.**
7. **Add 3 volumes of Gel Extraction Buffer to 1 volume of supernatant and mix. Check that the color of the mixture is yellow.**
   If the color of the mixture is orange or violet, add 10 ul 3 M sodium acetate, pH 5.0. The color of the mixture will turn yellow.
8. **Place a Nucleogen Gel Extraction Spin Column in a provided 2 ml collection tube.**
9. **To bind DNA, apply the sample to the Nucleogen Gel Extraction Spin Column and centrifuge for 30–60 s.**
10. **Discard flow-through and place Nucleogen Gel Extraction Spin Column back into the same collection tube.**
11. **To wash, add 750 ul Wash C Solution to column and centrifuge for 30–60 s.**
12. **Discard flow-through and place Nucleogen Gel Extraction Spin Column back in the same tube.**
    Centrifuge column for an additional 1 min at maximum speed.
    IMPORTANT: Residual ethanol from Wash C Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation.
13. **Place Nucleogen Gel Extraction Spin Column into a clean 1.5 ml microcentrifuge tube.**
14. **To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of the Nucleogen Gel Extraction Spin Column and centrifuge for 1 min. Alternatively, for increased DNA concentration, add 30 ul Elution Buffer to the center of the column, let stand for 1 min, and then centrifuge.**
    IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 ul from 50 ul elution buffer volume, and 28 ul from 30 ul.
    Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Ordering Information

Products		Contents	Cat. No.
RNA Stabilization Reagent		Tube (50 x 1.5 ml)	3502
		Tube (20 x 5 ml)	3205
		100 ml	3100
		250 ml	3250
Plasmid Purification Mini Kit			
	(200) for negative strain	200 preps	5112
	(200) for positive strain	200 preps	7112
Plasmid Purification Midi Kit	(10)	10 preps	6101
	(50)	50 preps	6105
	(100)	100 preps	6110
Plasmid Purification Maxi Kit	(6)	6 preps	7106
	(24)	24 preps	7124
	(50)	50 preps	7150
Gel Extraction Kit	(50)	50 preps	5215
	(200)	200 preps	5212
Highcon Gel Extraction Kit	(50)	50 preps	2215
	(200)	200 preps	2212
Bead Type (NaI) Gel Extraction Kit	(200)	200 preps	1232
	(400)	400 preps	1234
	(600)	600 preps	1236
PCR Purification Kits	(50)	50 preps	5315
	(200)	200 preps	5312
Highcon PCR Purification Kit	(50)	50 preps	2315
	(200)	200 preps	2312
DNA Clean-up Kits	(50)	50 preps	1415
	(200)	200 preps	1412
Genomic Blood Spin Mini Kit	(50)	50 preps	1515
	(200)	200 preps	1512
Genomic Blood Spin Midi Kit	(20)	20 preps	6520
	(50)	50 preps	6550
	(100)	100 preps	6500
Genomic Blood Spin Maxi Kit	(6)	6 preps	7506
	(24)	24 preps	7524
	(50)	50 preps	7550

Ordering Information

Products		Contents	Cat. No.
Genomic Cell / Tissue Spin Mini Kit	(50)	50 preps	1545
	(200)	200 preps	1542
Genomic Cell / Tissue Spin Midi Kit	(20)	20 preps	
	(50)	50 preps	
	(100)	100 preps	
Genomic DNA Isolation, Flexible		100 Isolation	1521
		500 Isolation	1525
		10 ml x 100 Isolation	
Apoptotic DNA Ladder Kit		50 preps	2505
96 PCR Purification Kit			
4 x 96 plates(binding, elution), buffer, tape			4304
25 x 96 plates(binding, elution), buffer, tape			4325
50 x 96 plates(binding, elution), buffer, tape			2 x 4325
96 Plasmid Purification Kit			
4 x 96 plates(clarification, binding, elution), buffer, tape			4104
25 x 96 plates(clarification, binding, elution), buffer, tape			4125
96 Genomic Blood Spin Kit			
4 x 96 plates(binding, elution), buffer, tape			
25 x 96 plates(binding, elution), buffer, tape			
96 Genomic Cell / Tissue Spin Kit			
4 x 96 plates(binding, elution), buffer, tape			
25 x 96 plates(binding, elution), buffer, tape			
50 x 96 plates(binding, elution), buffer, tape			2 x

www.nucleogen.com
Tel) 080-484-2007
031-315-6644
Fax) 031-312-2008