96 PCR Purification Kits

NucleoGen
BIOTECHNOLOGY

Contents

Kit Contents

Catalog No.	5315	5312
Number of preparations	50	200
Nucleogen 96 Binding Plates	50	200
96 Elution Plates	50	200
PCR Purification Buffer*	+	+
Wash A' Solution (concentrate)	+	+
Elution Buffer	+	+

* PCR Purification 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 Nucleogen PCR Purification 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 96 PCR Plate. Impurities are efficiently washed away, and the pure DNA is eluted with Elution Buffer or Water. The 96 PCR Purification Kits offer two handling options — as an alternative to processing in a centrifuge, they can now also be used on any commercial vacuum manifold.

Adsorption to 96 PCR Purification membrane

The 96 PCR Purification membrane is uniquely adapted to isolate DNA from aqueous solutions, and up to 10 ug DNA can bind to each well of 96 PCR Purification membrane.

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 96 PCR Plate. Salts are quantitatively washed away by the ethanol-containing Wash A' Solution. Any residual Wash A' 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 80 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, the way the buffer is applied to the membrane, and the incubation time of the buffer on the membrane. 100 ul of elution buffer is sufficient to completely cover the 96 PCR Purification membrane, ensuring maximum yield, even when not applied directly to the center of the membrane.
Elution with 60–80 ul requires the buffer to be added directly to the center of the membrane. Smaller elution buffer volumes give higher DNA concentrations in the eluate. For example, elution with 60 ul buffer will give a 1.5-times higher DNA concentration than elution with 80 ul buffer.

Important Notes

Please read the following notes before starting any of the 96 PCR Purification procedures.

Before equipment

- **Add ethanol(100%) to Wash A' Solution** before use (see bottle label for volume).
- All centrifuge steps are at 6,000 x g.

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

96 PCR Purification Kit Protocol
Using a Vacuum Manifold

This protocol is designed for the simultaneous purification of multiple samples of single-stranded DNA or double-stranded PCR products of 100 bp to 10 kb from primers, nucleotides, polymerases, and salts using the Nucleogen 96 PCR Purification Kit.

Please read "Important Notes" on pages 3 before starting.

1. **Add 150 ul of PCR Purification Buffer to 50 ul of PCR sample and mix.**
   Apply the samples to the wells of the 96 Binding Plate. Switch on vacuum source.
   It is not necessary to remove mineral oil or kerosene.

2. **After all liquid has passed through the membrane, switch off the vacuum source.**

3. **Add 300 ul of Wash A' Solution to each well of the 96 Binding Plate.**
   Switch on vacuum source.

4. **Repeat step 3.**

5. **After Wash A' Solution in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.**
   IMPORTANT: This step removes residual Wash A' Solution from the membrane. Residual ethanol, from Wash A' Solution, may inhibit subsequent enzymatic reactions, such as sequencing.

6. **Vigorously tap the 96 PCR Binding Plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the 96 Binding Plate with clean absorbent paper.**
   This step removes residual Wash A' Solution from around the outlet nozzles and collars of the 96 Binding Plate. Residual ethanol, from Wash A' Solution, may inhibit subsequent enzymatic reactions, e.g., sequencing.

7. **place the a 96 Binding plate on top of a 96 Elution plate.**

8. **To elute, add 80 ul of Elution Buffer (10 mM Tris, pH 8.5) or nuclease-free water to the center of each well of the 96 Binding plate, let stand for 1 min.**

9. **Switch on vacuum source.**
   Please note that the average volume of eluate is 60 ul using 80 ul of Elution Buffer.
   Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and pH 8.5. When using water to elute, 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.

96 PCR Purification Kit
Using a Centrifuge

Please read "Important Notes" on pages 3 before starting.

1. **Place Nucleogen 96 PCR Plate on top of a Square-Well Block (not include). Mark the Nucleogen 96 PCR Plate for later identification.**

2. **Add 150 ul of PCR Purification Buffer to 50 ul of PCR sample and mix. Pipet the samples into the wells of the Nucleogen 96 PCR Plate.**
   It is not necessary to remove mineral oil or kerosene.

3. **Load each Square-Well Block and Nucleogen 96 PCR Plate onto the carrier then place in the rotor bucket. Centrifuge at 6,000 x g for 4 min.**

4. **Empty the Square-Well Block. Add 300 ul of Wash A' Solution to each well. Centrifuge at 6,000 x g for 4 min.**

5. **Repeat step 4.**

6. **Incubate for 10 min at 70°C in an incubator or oven to dry the membrane, or centrifuge at 6,000 x g for 4 min.**
   IMPORTANT: This step removes residual Wash A' Solution from the membrane. Residual ethanol, from Wash A' Solution, may inhibit subsequent enzymatic reactions, such as sequencing.

7. **place the a 96 Binding plate on top of a 96 Elution plate.**

8. **To elute, add 80 ul of Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the 96 Binding Plate, allow to stand for 1 min.**

9. **Centrifuge at 6,000 x g for 4 min.**
   Please note that the average volume of eluate is 60 ul using 80 ul of Elution Buffer.
   Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and pH 8.5. When using water to elute, 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.

Troubleshooting Guide

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

	Comments and suggestions
Low or no yield	
a) Wash A' Solution did not contain ethanol	Ethanol must be added to Wash A' Solution (concentrate) before use. Repeat procedure with correctly prepared Wash A' 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 (60 ul).
DNA does not perform well, e.g., in ligation reactions	
a) Salt concentration in eluate too high	Modify the wash step by incubating the 96 Binding Plate for 5 min at room temperature after adding 300 ul of Wash A' Solution, then switch on vacuum source or centrifuge.
b) Elution contains residual ethanol	Incubate for 10 min at 70°C in an incubator or oven to dry the membrane, or centrifuge at 6,000 x g for 4 min.
c) Elution contains primer-dimers	Primer-dimers formed are longer than 20 bp, and are not completely removed. After the binding step, wash the 96 Binding Plate with 300 ul of a 35% guanidine hydrochloride aqueous solution. Continue with the Wash A' Solution wash step and the elution step as in the protocol.
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.

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

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