PCR Purification Kits

NucleoGen
BIOTECHNOLOGY

Contents

Kit Contents

Catalog No.	5315	5312
Number of preparations	50	200
Nucleogen Spin Columns	50	200
Collection tubes (2 ml)	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 column. Impurities are efficiently washed away, and the pure DNA is eluted with Elution Buffer or Water. The PCR Purification 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 PCR Purification membrane

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

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 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 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 PCR Purification 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 PCR Purification column is incubated for 1 min with 30 ul of elution buffer, than if it is eluted in 50 ul without incubation.

Important Notes

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

Before equipment

- **Add ethanol(100%) to Wash A' Solution** before use (see bottle label for volume).
- All centrifuge steps are at 13,000rpm (~17,900 x g) in a conventional tabletop 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

PCR Purification Kit Protocol

Using a Microcentrifuge

Please read "Important Notes" on pages 13 before starting.

This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions. For cleanup of other enzymatic reactions. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using spin columns in a microcentrifuge.

1. **Add 5 volumes of PCR Purification Buffer to 1 volume of the PCR sample and mix**.
   For example, add 500 ul of PCR Purification Buffer to 100 ul PCR sample (not including oil).
2. **Place a spin column in a provided 2 ml collection tube.**
3. **To bind DNA, apply the sample to the column and ecntrifuge for 30-60 sec.**
4. **Discard flow-through and place the column back in the same tube.**
   Collection tubes are re-used to reduce plastic waste.
5. **To wash, add 750 ul Wash A' Solution to the column and centrifuge for 30-60 sec**.
6. **Discard flow-through and place the column back in the same tube.**
   Centrifuge the column for an additional 1 min.
   IMPORTANT: Residual ethanol from Wash A' Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation.
7. **Place column in a clean 1.5 ml microcentrifuge tube.**
8. **To elute DNA**, **add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the column membrane and centrifuge the column for 1 min.**
   Alternatively, for increased DNA concentration, add 30 ul Elution Buffer to the center of each membrane, let the columns stand for 1 min, and then centrifuge.

Using a Vacuum Manifold

Please read "Important Notes" on pages 13 before starting.

1. **Add 5 volumes of PCR Purification Buffer to 1 volume of the PCR sample and mix**.
   For example, add 500 ul of PCR Purification Buffer to 100 ul PCR sample (not including oil).
2. **Prepare the vacuum manifold and PCR Purification columns:**
   - Insert each spin column into a luer connector on the Luer Adapter(s) in the manifold.
3. **To bind DNA, load the samples into the spin columns by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.**
   The maximum loading volume of the column is 800 ul. For sample volumes greater than 800 ul simply load again.
4. **To wash, add 750 ul of Wash A' Solution to each spin column and apply vacuum.**
5. **Transfer each spin column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at 13,000 rpm (~17,900 x g).**
   IMPORTANT: This spin is necessary to remove residual ethanol (Wash A' solution).
6. **Place each spin column into a clean 1.5 ml microcentrifuge tube.**
7. **To elute DNA, add 50 ul of Elution Buffer (10 mM Tris·Cl, pH 8.5) or H_2O to the center of each membrane, and centrifuge the columns for 1 min at 13,000 rpm (~17,900 x g). Alternatively, for increased DNA concentration, add 30 ul Elution Buffer to the center of each membrane, let the columns stand for 1 min, and then centrifuge.**
   IMPORTANT: Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of bound DNA.

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 (30 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 column for 5 min at room temperature after adding 750 ul of Wash A' 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 contains primer-dimers	Primer-dimers formed are longer than 20 bp, and are not completely removed. After the binding step, wash the spin column with 750 ul of a 35% guanidine hydrochloride aqueous solution (35 g in 100 ml). 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.

Additional Protocol

Purification of DNA fragments from dye-labeled reactions using the Nucleogen PCR Purification Kit

This protocol is designed to purify single- or double-stranded DNA fragments from dye-labeled reactions. Fragments ranging from 100 bp to 10 kb are purified from dye-labeled nucleotides (e.g. Cy3-dNTP or Cy5-dNTP), primers, polymerases, and salts using Nucleogen Spin columns in a microcentrifuge.

Please read "Important Notes" on pages 13 before starting.

- Recoveries for fragments less than 200 nucleotides may be reduced.

1. **Add 5 volumes of PCR Purification Buffer to 1 volume of the sample and mix.** It is not necessary to remove mineral oil or kerosene.
   For example, add 500 ul of PCR Purification Buffer to 100 ul sample (not including oil).

2. **Place a PCR Purification Spin column in a provided 2 ml collection tube.**

3. **To bind DNA, apply the sample to the Spin column and centrifuge for 30–60 s.**

4. **Discard flow-through. Place the Spin column back into the same tube.**
   Collection tubes are re-used to reduce plastic waste.

5. **To wash, add 0.75 ml of a 35% guanidine hydrochloride aqueous solution (not include) and centrifuge for 30-60 s.**

6. **Discard flow-through and place the Spin column back into the same tube.**

7. **Add 750 ul of Wash A' Solution to the Spin column and centrifuge for 30–60 s.**

8. **Discard flow-through and place the Spin column back in the same tube.**
   Centrifuge the column for an additional 1 min at maximum speed.
   IMPORTANT: Residual ethanol from Wash A' Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation.

9. **Place Spin column in a clean 1.5 ml microcentrifuge tube.**

10. **To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of the PCR Purification membrane and centrifuge the column for 1 min.** Alternatively, for increased DNA concentration, add 30 ul elution buffer to the center of the PCR Purification membrane, let the column stand for 1 min, and then centrifuge.
    IMPORTANT: Ensure that the elution buffer is dispensed directly onto the PCR Purification membrane for complete elution of bound DNA. The average eluate volume is 48 ul from 50 ul elution buffer volume.
    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

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