

PuroSPIN™ PCR Product Purification Kit

#NK105-50, #NK105-250

Product Manual

Version 2.2

Kit Contents

Component	50 Preps (#NK105-50)	250 Preps (#NK105-250)
Binding Buffer BB-PP	50 mL	220 mL
Wash Buffer WB-PP (<i>after Ethanol addition</i>)	120 mL	2 x 225 mL
Elution Buffer EB	15 mL	50 mL
Water, Nuclease-free	15 mL	50 mL
PuroSPIN™ MINI Spin Columns	50	250
Collection Tubes (2 mL)	50	250

Storage

All components can be stored at room temperature for at least 1 year.

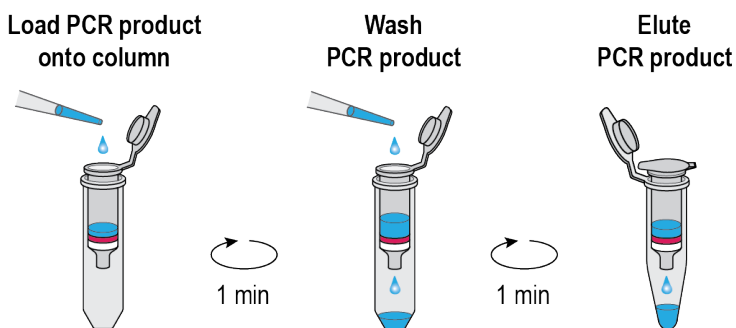
Additional materials and equipment required

- 95-100% Ethanol
- 1.5 mL microcentrifuge tubes
- Microcentrifuge (with 12,000g capability)
- Appropriate personal protective equipment (gloves, lab coat)

Kit Description

PuroSPIN™ PCR Purification Kit is designed to provide simple and easy-to-use method for purification of PCR DNA products (100 bp – 10 kb) from enzymatic reactions. The purification technology is based on nucleic acid binding to silica spin-columns, which allows efficient removal of protein, salt and other contaminants. Silica spin columns offer faster, simpler and safer alternative to traditional nucleic acid purification techniques such as phenol-chloroform extraction. PuroSPIN™ buffer formulations produce highly purified DNA product for downstream applications, such as sequencing, ligation, transfection, Southern blotting, qPCR, or microarray analysis. PuroSPIN™ columns can bind up to 200 µg of DNA product.

Experimental Workflow



Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-PP**:
 - **#NK105-50** (50 preps): add **90 mL** of Ethanol
 - **#NK105-250** (250 preps): add **170 mL** of Ethanol



- **Binding Buffer BB-PP** contains **guanidine hydrochloride**. It is a known irritant that is harmful if inhaled, swallowed, or contacted with skin. Wear appropriate protective equipment when working with this reagent.
- If precipitate is observed in the **Binding Buffer BB-PP** re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.


Protocol

Step	Procedure
1	Add 4 volumes of Binding Buffer BB-PP to 1 volume of DNA product. For example, add 400 μL of Binding Buffer BB-PP to 100 μL of DNA sample.
3	Transfer up to 750 μL of the sample into the PuroSPIN™ MINI Spin Column . Centrifuge at 12,000g for 1 minute to allow DNA to bind to the column. Discard the flow-through. <i>NOTE: If the total sample volume exceeds 750 μL, repeat <u>Step 3</u> until all of the sample has been loaded onto the column.</i>
4	Add 700 μL of Wash Buffer WB-PP and centrifuge at 12,000g for 1 minute. Discard the flow-through.
5	Add another 700 μL of Wash Buffer WB-PP and centrifuge at 12,000g for 1 minute. Discard the flow-through.
6	Centrifuge at 12,000g for 1 minute to remove the residual washing buffer.
7	Transfer the column into a clean 1.5 mL microcentrifuge tube.
8	Add 30-100 μL of Elution Buffer EB or nuclease-free water . Incubate for 2 minutes at room temperature. <i>NOTE: Smaller volume will yield higher concentration DNA product, while larger volume will maximize the total amount of eluted DNA. The eluted DNA amount can be further increased by repeating <u>Steps 8 and 9</u> using another 100 μL of Elution Buffer EB or nuclease-free water.</i>
9	Centrifuge the column at 12,000g for 1 minute to collect the purified product.

Troubleshooting

Problem	Possible causes and solutions
Low or no product yield	<p>Ethanol was not added to the Wash Buffer WB-PP. Make sure the indicated amount of 96-100% Ethanol is added to the Wash Buffer WB-PP before use.</p> <p>Isopropanol was not added to the sample. Make sure the indicated amount of isopropanol is added to the sample before loading it onto the column.</p> <p>Incorrect elution buffer. Make sure to use low salt and high pH (8 – 8.5) elution buffer. Make sure to pipette that elution buffer directly onto the center of the membrane.</p> <p>Low starting amount of PCR product. Verify the product was successfully amplified during the PCR reaction by visualizing it using agarose gel electrophoresis.</p>

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Binding Buffer BB-PP		<p>Contains <i>guanidine hydrochloride</i></p> <ul style="list-style-type: none"> • Harmful if swallowed. • Irritating to eyes and skin. • Do not breathe vapors and fumes • Wear suitable protective clothing and gloves. • In case of contact with eyes immediately wash with plenty of water and seek medical advice. • If swallowed, seek medical advice and contact poison control center.

This product is intended solely for research use only. Full material safety data sheet document is available at www.lunanano.com.

For further information and other products please refer to www.lunanano.com.