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PCR Purification 96-Well Kit — Product Insert

Product # 24800

Norgen's PCR Purification 96-Well Kit provides a rapid method for the high-throughput purification of amplified DNA products from PCR mixes. This kit is able to effectively remove PCR by-products including primers, dimers, enzymes, unincorporated nucleotides and mineral oil from the desired PCR product. The purified PCR products are fully compatible with restriction enzyme digestion, ligation into vectors, labeling and sequencing. This kit can also be used as an alternative to organic extraction and ethanol precipitation to clean up various enzymatic reactions.

Norgen's Purification Technology

Purification is based on 96-well column chromatography. Norgen's 96-well plate binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The PCR product is first mixed with 5 volumes of the provided Binding Buffer C (please see the flow chart on page 3). It is not necessary to remove the mineral oil from the PCR sample, as it will be removed during the purification process. Next, the sample is loaded onto the 96-Well Plate using either a vacuum manifold or centrifugation. Norgen's plate binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the plate while most of the primers, dimers and other contaminants will flowthrough. The bound DNA is then washed twice using the provided Wash Solution A in order to remove any remaining impurities, and the purified PCR products are eluted with the Elution Buffer B.

Specifications

Kit Specifications	
Binding Capacity Per Well	15 µg
Maximum Loading Volume Per Well	500 µL
Size of DNA Purified	100 – 15,000 bp
Average DNA Recovery	> 90%
Average Primer Removal	> 90%
Minimum Elution Volume	50 µL
Time to Complete 96 Purifications	20 minutes

Advantages

- Fast and easy processing using either a vacuum manifold or centrifugation
- Purification from all PCR by-products, including primers and dimers
- Average primer removal is greater than 90%
- High recovery of PCR products; DNA recovery is greater than 90% of the input DNA

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Kit Components

Component	Product # 24800 (192 preps)
Binding Buffer C	3 x 30 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	2 x 15 mL
96-Well Plate	2
Adhesive Tape	4
96-Well Collection Plate	2
96-Well Elution Plate	2
Product Insert	1

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Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Binding Buffer C** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- For ***Vacuum Format***:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads
- For ***Centrifuge Format***:
  - Centrifuge with rotor for 96-well plate assembly
- 95 - 100% ethanol

Procedure

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Flow Chart

Procedure for the Rapid 96-Well Purification of Amplified DNA Products from PCR Mixes

Add 5 Volumes of Binding Buffer C to PCR Reaction

Bind DNA

Wash Twice with
Wash Solution A

Elute with Elution
Buffer B

Purified PCR Products

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.
- The purification of PCR reactions can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section A. For purification using centrifugation, please follow the procedure outlined in Section B.

A. Purification of PCR Products Using A Vacuum Manifold

1. Sample Preparation

a. Add 5 volumes of **Binding Buffer C** directly to each tube containing a PCR reaction. The maximum volume of PCR reaction that can be processed is 75 μL. Mix by pipetting up and down or vortexing.

Note: It is not necessary to remove the mineral oil from the PCR sample, as it will be removed during the purification process.

2. Binding DNA to 96-Well Plate

a. Assemble the 96-Well Plate and the vacuum manifold according to manufacturer's recommendations.

Note: The provided 96-Well Collection Plate can be used as the collection/waste tray if desired.

b. Apply up to 500 μL of the PCR reaction mix into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

Note: Ensure that all of the reaction mix from each well has passed through into the collection/waste tray. If the entire reaction mix volume has not passed, apply vacuum for an additional 2 minutes.

3. Washing Bound DNA

a. Apply 400 μL of **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the 96-Well Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a second time.
d. Pat the bottom of the 96-Well Plate dry. Reassemble the 96-Well Plate and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
e. Turn off vacuum and ventilate the manifold.

4. DNA Elution

a. Remove the lid from the provided Elution Plate. Replace the collection/waste tray in the vacuum manifold with the Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
b. Add 75 µL of **Elution Buffer B** to each well of the plate.
c. Apply vacuum for 2 minutes.

5. Storage of DNA

Use the provided adhesive tape and/or lid to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Purification of PCR Products Using Centrifugation

1. Sample Preparation

a. Add 5 volumes of **Binding Buffer C** directly to the tube containing the PCR reaction. The maximum volume of PCR reaction that can be processed is 75 µL. Mix by pipetting up and down or vortexing.

Note: It is not necessary to remove the mineral oil from the PCR sample, as it will be removed during the purification process.

2. Binding DNA to 96-Well Plate

a. Place the 96-Well Plate on top of a provided 96-Well Collection Plate.
b. Apply up to 500 µL of the PCR reaction mix into each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.
c. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. DNA Wash

a. Apply 400 µL of **Wash Solution A** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the bottom plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.
c. Repeat steps **3a** and **3b** to wash column for a second time.

d. Pat the bottom of the 96-Well Plate dry. Reassemble the 96-Well Plate and the bottom plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 5 minutes in order to completely dry the plate.

4. DNA Elution

a. Remove the lid from the provided Elution Plate. Stack the 96-Well Plate on top of the Elution Plate.
b. Add 75 µL of **Elution Buffer B** to each well of the 96-Well Plate.
c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

5. Storage of DNA

Use the provided adhesive tape and/or lid to seal the Elution Plate. The purified DNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA recovery	Binding of DNA to the plate was inefficient	Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of **Binding Buffer C** was used for the volume of the PCR reaction.
	The appropriate amount of ethanol was not added to the **Wash Concentrate**	The **Wash Solution A** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution A** was prepared using the correct amount of ethanol.
	Binding Buffer C was not completely removed in the wash step.	Traces of salt left on the plate from the binding step may interfere with the elution of the DNA. Ensure that the column is washed with the **Wash Solution A**.
	Proper Elution Buffer was not used	The provided **Elution Buffer B** has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8.
	Insufficient vacuum	Ensure that a vacuum pressure of at least -650 mbar of -25 in. Hg is developed. Insufficient vacuum will result in low eluate volumes, or eluate volumes varying between wells.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	Insufficient washing of plate with bound DNA	Traces of salt from the binding step may remain in the sample if the column is not properly washed with the **Wash Solution A**. Ensure that the column is spun for 2 minutes during the washing step. Salt may interfere with downstream applications, and thus must be washed from the column.
	Eluted DNA contains residual ethanol	Ensure that the dry spin is performed after the second DNA wash to remove all traces of ethanol (Step **3d**).
Clogged Wells in Plate	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.

Related Products	Product #
PCR Purification Kit	14400
DNA Gel Extraction Kit	13100

Technical Support
Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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