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Sequencing Reaction Clean-Up 96-Well Kit — Product Insert

Product # 34400

Norgen's Sequencing Reaction Clean-Up 96-Well Kit provides a rapid, simple and efficient procedure for the high-throughput purification and clean-up of sequencing and various other enzymatic reactions including restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations. The kit is used to remove reaction contaminants including dye terminators, salts, enzymes, excess primers and primer dimers. Contaminants are undesirable as they can interfere with many downstream applications including sequencing, RFLP, restriction enzyme digestions and ligation. Purification is based on column chromatography without the use of phenol, chloroform or alcohol precipitation. The kit provides a high quality product with up to 90% recovery.

Norgen's Purification Technology

Purification is based on 96-well column chromatography. The sequencing extension product or enzymatic reaction is preferentially purified from contaminants such as salt, enzymes and dye terminators without the use of phenol or chloroform. The process involves first mixing the sample containing the extension product or enzymatic reaction with Binding Buffer C (please see the flow chart on page 3). Next, the sample is loaded onto the 96-Well Plate using either a vacuum manifold or centrifugation. Only the nucleic acids will bind to the plate, while the contaminating dye terminators, excess primers, primer dimers, salts or proteins will be removed in the flowthrough. The bound nucleic acid is then washed with the provided Wash Solution A in order to remove any remaining impurities. The purified product is of the highest integrity to be used in sequence analysis and other downstream applications.

Specifications

Kit Specifications	
Binding Capacity Per Well	15 μg
Maximum Loading Volume Per Well	500 μL
Average DNA Recovery	Up to 90%
Minimum Elution Volume	50 μL
Time to Complete 96 Purifications	20 minutes

Advantages

- **Fast and easy processing** - Rapid 96-well format to be processed by using either a vacuum manifold or centrifugation.
- **Purification from all sequence cycling by-products** - Remove all dye terminators, primer-dimers, primers, enzymes and salts.
- **Purification of all types of enzymatic reactions** - Purify DNA from different enzymatic reactions including restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations.
- **High recovery** - Recovery of up to 90% of input amount.
- **Complete column purification** – The extension product or enzymatic reaction is column cleaned without the use of phenol, chloroform or alcohol precipitation.
- **High integrity product** – The cleaned extension product or enzymatic reaction is ready to be used in various downstream applications.

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 # 34400 (192 preps)
Binding Buffer C	3 x 30 mL
Wash Solution A	2 x 20 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	2

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.

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
- 96 - 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:

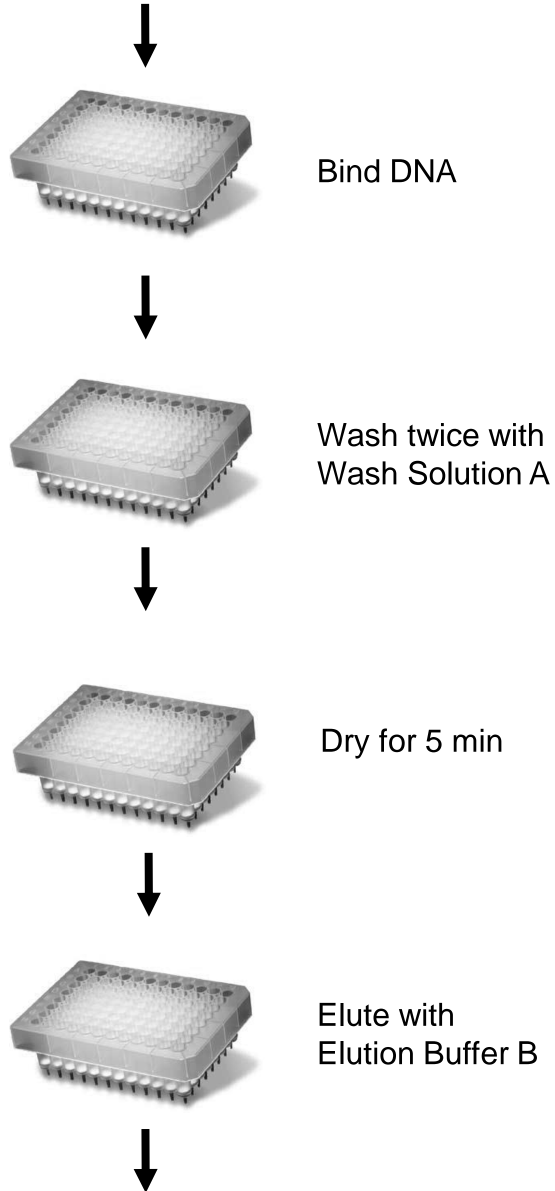
$$\mathrm{RPM} = \sqrt{\frac{\mathrm{RCF}}{(1.118 \times 10^{-5})\,(r)}}$$

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 Clean-Up of Sequencing Extension Products

Add 5 volumes of Binding Buffer C to the extension product
or enzymatic reaction and mix.



Purified extension product or enzymatic reaction

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 80 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 100 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- The purification the extension product 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 Extension Products Using A Vacuum Manifold

1. Sample Preparation

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

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 mixture 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 plate 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.

B. Purification of Extension Products Using Centrifugation

1. Sample Preparation

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

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 mixture 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 plate 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.

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 extension product.
	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.
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 #
Sequencing Reaction Clean-Up Kit	34500
PCR Purification Kit	14400
DNA Gel Extraction Kit	13100

Technical Support
Contact our Technical Support Team between the hours of 8:30 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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