3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Sequencing Reaction Clean-Up Kit

Product Insert

Product # 34500

Norgen's Sequencing Reaction Clean-Up Kit provides a rapid, simple and efficient procedure for the 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 spin-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 spin column chromatography. The sequencing extension product or other enzymatic reaction products are 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). The mixture is then loaded onto a spin-column. Norgen's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the nucleic acids will bind to the column, while the contaminating dye terminators, excess primers, primer dimer, 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 then eluted with the Elution Buffer B. The purified product is of the highest integrity to be used in sequence analysis and other downstream applications.

The kit is designed to process 50 samples.

Specifications:

Kit Specifications	
Column Binding Capacity	13 µg
Maximum Column Loading Volume	600 µL
Maximum Amount of Starting Material	25 µg
Time to Complete 10 Purifications	10 minutes
Minimum Elution Volume	10 µL

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment.

Advantages:

- **Fast and easy processing** - Rapid spin-column format processes 10 samples in 10 minutes.
- **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.

Kit Components:

Component	Contents
Binding Buffer C	30 mL
Wash Solution A	12 mL
Elution Buffer B	8 mL
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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*.

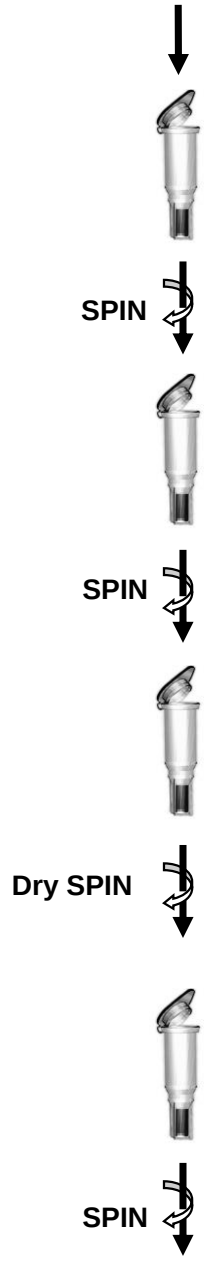
Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- Nuclease-free water
- 96 - 100% ethanol

Flow Chart

Procedure for the Clean-Up of Sequencing Extension Products and Enzymatic Reactions

Add 5 volumes of Binding Buffer C to the extension product
or ezymatic reaction



Vortex to mix

Bind to column

SPIN 14,000 x g, 1 min

Wash with 500 µL
Wash Solution A

SPIN 14,000 x g, 1 min

Dry SPIN 14,000 x g, 2 min

Elute with 50 µL
Elution Buffer B

SPIN 14,000 x g, 1 min

Purified Extension Product or Enzymatic Reaction Product

Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. 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.

Notes Prior to Use

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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 48 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.

1. Sample Preparation and Binding to Column

a. Add 5 volumes of **Binding Buffer C** directly to the tube containing the extension product or enzymatic reaction and mix well. Vortex and pulse-spin briefly in microcentrifuge to aid in mixing.

b. Obtain a spin column and assemble with a collection tube. Apply the sample to the column and centrifuge for 1 minute at 8,000 x g (~8,000 RPM). The maximum volume that the reservoir can accommodate during each spin is 650 µL. If the sample volume exceeds this, repeat as necessary until the entire sample has been processed.

Note: It is important that the sample be added to the center of the column bed and not onto the side of the tube.

c. Discard the flowthrough and reassemble the spin column with its collection tube.

2. Column Wash

a. Apply 500 µL of **Wash Solution A** to column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).

b. Discard the flowthrough and reassemble the spin column with its collection tube.

c. Spin the column for 2 minutes at 14,000 x g (~14,000 RPM) in order to thoroughly dry the column. Discard the collection tube.

3. Elution of Clean Extension Product

a. Assemble the column with one of the provided 1.7 mL Elution tubes.

b. Add 50 µL of **Elution Buffer B** (minimum 10 µL) to the center of the column bed. Centrifuge and let stand at room temperature for 1 minute

c. Centrifuge at **14,000 x g (~14,000 RPM) for 2 minutes**.

d. (Optional): An additional elution can be performed by repeating the previous steps**.** This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

Troubleshooting Guide

Problem	Possible cause	Solution and Explanation
Poor DNA recovery	Binding of DNA to the column 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 column 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.
	Elution Buffer B was not placed directly onto the column bed	It is important that the **Elution Buffer B** be placed directly onto the column bed, as this helps to increase recovery by ensuring an even passing of the buffer through the column. Do not pipette the **Elution Buffer B** onto the side of the column.
DNA does not perform well in downstream applications	Insufficient washing of column 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.

Related Products	Product #
Sequencing Reaction Clean-Up 96-Well Kit	34400
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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