NORGEN BIOTEK CORPORATION

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

# PCR Purification Kit

# Product Insert

**Product # 14400, 45700**

Norgen's PCR Purification Kit enables the rapid 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 spin column chromatography. Norgen's column 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 applied to one of the provided spin columns through centrifugation. Norgen's column binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while most of the primers, dimers and other contaminants will flowthrough the column. The bound DNA is then washed twice using the provided Wash Solution A in order to remove any remaining impurities, and the purified PCR product is eluted with the Elution Buffer B.

## Specifications

| Kit Specifications | |
|---|---|
| Column Binding Capacity | 10 µg |
| Size of DNA Purified | 100 – 15,000 bp |
| Average DNA Recovery | > 90% |
| Average Primer Removal | > 90% |
| Minimum Elution Volume | 30 µL |
| Time to Complete 10 Purifications | 15 minutes |

## Advantages

- Fast and easy processing using a rapid spin-column format
- 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. These reagents should remain stable for at least 1 year in their unopened containers.

**Kit Components**

| Component | Product # 14400 (50 samples) | Product # 45700 (250 samples) |
|---|---|---|
| Binding Buffer C | 30 mL | 5 x 30 mL |
| Wash Solution A | 12 mL | 2 x 20 mL |
| Elution Buffer B | 8 mL | 2 x 30 mL |
| Spin Columns | 50 | 250 |
| Collection Tubes | 50 | 250 |
| Elution tubes (1.7 mL) | 50 | 250 |
| Product Insert | 1 | 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*.

The Binding Buffer C contains guanidine hydrochloride and isopropanol, 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**

- Benchtop microcentrifuge
- 96 - 100% ethanol

# Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

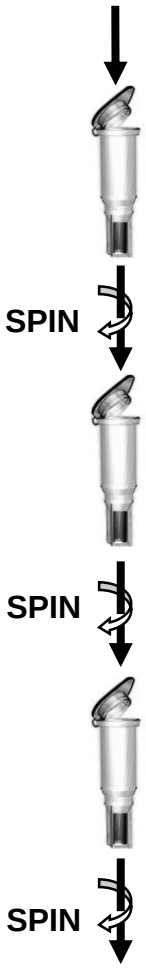
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

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 Purification of Amplified DNA Products from PCR Mixes

Add 5 Volumes of Binding Buffer C to PCR Reaction



Bind to Column

**SPIN**

Wash with Wash Solution A

**SPIN**

Elute with Elution Buffer B

**SPIN**

**Purified PCR Product**

**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.
- **Product # 14400 (50 Prep Kit):** 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.
- **Product # 45700 (250 Prep Kit):** Prepare a working concentration of **Wash Solution A** by adding 80 mL of 96 - 100% ethanol (to be provided by the user) to each of the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 100 mL for each bottle. The labels on the bottles have 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 PCR reaction and mix well. Vortex and pulse-spin briefly in microcentrifuge to aid in mixing.

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

**b.** Assemble a spin column with one of the provided collection tubes. 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 750 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. Washing Bound DNA**

**a.** Apply 500 µL of **Wash Solution A** to column and centrifuge for 1 minute at 10,000 x g (~10,000 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 DNA**

**a.** Assemble the column with one of the provided 1.7 mL **Elution tubes**.
**b.** Add 50 µL of **Elution Buffer B** to the center of the column bed. It is important that the **Elution Buffer B** be placed directly onto the column bed, and not onto the side of the column to obtain the best DNA recovery.
**c.** Let stand at room temperature for 1 minute.
**d.** Centrifuge for 2 minutes at **14,000 x g (~14,000 RPM).**

(Optional): An additional elution can be performed by repeating Steps **3b to 3d.** 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 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 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 # |
|---|---|
| DNA Gel Extraction Kit | 13100 |
| Sequencing Reaction Cleanup Kit | 34500 |

**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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362