

## Enzymatic Reaction Clean-Up Kit Product # 19900

## Product Insert

Norgen's Enzymatic Reaction Clean-Up Kit enables the rapid purification of DNA from various enzymatic reaction mixes. This kit is able to purify DNA from different enzymatic reactions including restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations. The purified DNA can be used in a number of downstream applications including restriction enzyme digestions and ligations.

### Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The enzymatic reaction mixture containing the DNA of interest is first mixed with 5 volumes of the provided Binding Solution (please see the flow chart on page 3). Next, the sample is applied to one of the provided spin columns through centrifugation. Norgen's resin binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while any enzymes, reaction by-products and other contaminants will flowthrough the column. The bound DNA is then washed twice using the provided Wash Solution in order to remove any remaining impurities, and the purified PCR product is eluted with the Elution Buffer.

### Specifications

Kit Specifications	
Column Binding Capacity	25 µg
Size of DNA Purified	100 – 15,000 bp
Average DNA Recovery	> 90%
Minimum Elution Volume	30 µL
Maximum Volume Input	100 µL
Time to Complete 10 Purifications	15 minutes

### Advantages

- Fast and easy processing using a rapid spin-column format
- Purification from all types of enzymatic reactions, including restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations
- High DNA recovery; DNA recovery is greater than 90% of the input amount

## Kit Components

Component	Product # 19900 (50 samples)
Binding Solution	25 mL
Wash Solution	8 mL
Elution Buffer	6 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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## 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.

## 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](http://www.norgenbiotek.com).

The Binding Solution 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.

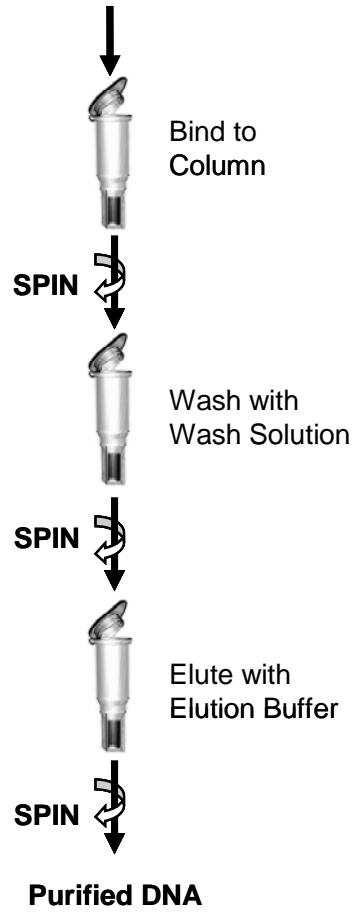
## Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96 - 100% ethanol

## Flow Chart

Procedure for the Rapid Purification of DNA from Enzymatic Reaction Mixes

Add 5 Volumes of Binding Solution to Enzymatic Reaction



## Procedure

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:

- 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** by adding 32 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution**. This will give a final volume of 40 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 Solution** directly to the tube containing the enzymatic reaction and mix well. Vortex and pulse-spin briefly in microcentrifuge to aid in mixing.
- b. Assemble a spin column with a provided collection tube. Apply the sample to the column assembly and centrifuge for 1 minute. The maximum volume that the reservoir can accommodate during each spin is 600  $\mu$ L. 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 resin 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  $\mu$ L of **Wash Solution** to column and centrifuge for 2 minutes at 16,000 x  $g$ .
- b. Ensure that all the **Wash Solution** has passed through the column and that the column is dry. Spin for an additional minute if necessary
- c. Discard the flowthrough and collection tube.

### 3. Elution of Clean DNA

- a. Assemble the column with one of the provided 1.7 mL **Elution tubes**.
- b. **For DNA fragments 1000 bp and larger:**  
Add 50  $\mu$ L of **Elution Buffer** to the center of the resin bed. It is important that the **Elution Buffer** be placed directly onto the resin bed, and not onto the side of the column to obtain the best DNA recovery. Centrifuge for 2 minutes at **2,000 x g**. Centrifuge the elution for an additional 1 minute at **14,000 x g**.

**For DNA fragments smaller than 1000 bp:**

Add 50  $\mu$ L of **Elution Buffer** to the center of the resin bed. Centrifuge the elution for 1 minute at **14,000 x g**

- c. (Optional): An additional elution can be performed by repeating steps **3a** and **3b**. 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 <b>Binding Solution</b> was used for the volume of the enzymatic reaction.
	The appropriate amount of ethanol was not added to the <b>Binding Concentrate</b>	The <b>Binding Solution</b> has been specifically designed to contain the appropriate amount of components. Ensure that the correct amount of ethanol was added to the <b>Binding Solution</b> .
	The appropriate amount of ethanol was not added to the <b>Wash Concentrate</b>	The <b>Wash Solution</b> has been specifically designed to contain the appropriate amount of components. Ensure that the <b>Wash Solution</b> was prepared using the correct amount of ethanol.
	<b>Binding Solution</b> 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 <b>Wash Solution</b> .
	Proper <b>Elution Buffer</b> was not used	The provided <b>Elution Buffer</b> has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8.
	<b>Elution Buffer</b> was not placed directly onto the resin	It is important that the <b>Elution Buffer</b> be placed directly onto the resin, as this helps to increase recovery by ensuring an even passing of the buffer through the resin. Do not pipette the <b>Elution Buffer</b> onto the side of the column.
DNA does not perform well in downstream applications	Insufficient washing of resin with bound DNA	Traces of salt from the binding step may remain in the sample if the column is not properly washed with the <b>Wash Solution</b> . 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.

<b>Related Products</b>	<b>Product #</b>
DNA Gel Extraction Kit	13100
PCR Purification Kit	14400

### **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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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