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DNA Clean-Up and Concentration Micro-Elute Kit Product Insert

Product #67200

Norgen's DNA Clean-Up and Concentration Micro-Elute Kit provides a rapid method for the purification, cleanup and concentration of up to 40 μg of DNA from PCR reactions, endonuclease digestions as well as from DNA modification reactions, labeling reactions and more. Plasmids <10,000 bp in size and genomic DNA may also be concentrated. The minimum recommended elution volume is 8 μL, which enables the concentration of small amounts of all sizes of DNA. The DNA is preferentially purified from other reaction components such as proteins, nucleases and free nucleotides or other reaction components. The purified DNA is free of salts and contaminants and is of the highest integrity ready for use in PCR, DNA sequencing, ligation reactions, endonuclease digestion, radiolabeling, arrays and more. Preparation time for a single sample is approximately 20 minutes, and each kit contains sufficient materials for 50 preparations.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The DNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the DNA samples or enzymatic reactions containing DNA with Buffer RL. Ethanol is then added and the mixture is loaded onto an activated spin-column specifically designed for small elution volumes. Norgen's resin binds DNA in a manner that depends on ionic concentrations. Thus only the DNA will bind to the column, while the contaminating proteins or free nucleotides will be removed in the flowthrough. The bound DNA is then washed three times with the provided Wash Solution A in order to remove any remaining impurities. The purified DNA is then eluted into a small volume (8 –15 μL) using Elution Buffer F.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	40 μg of DNA
Size of DNA Purified	50 bp to 10,000 bp
Maximum Volume of Starting Material	200 μL
Minimum Elution Volume	8 μL
Time to Complete 10 Purifications	20 minutes
Average Recovery	≥ 90%

Advantages

- Concentration of small amounts of DNA into 8 to 15 μL
- Ideal for concentrating DNA from PCRs and other enzymatic and labelling reactions
- No need for organic denaturants or chloroform
- Cleanup of plasmids or DNA isolated using other DNA isolation methods
- Fast and easy processing using rapid spin-column format
- Suitable for DNA from 50 bp to 10,000 bp

Kit Components

Component	Product #67200 (50 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Buffer F	6 mL
Column Activation Solution	30 mL
Micro-Elute DNA Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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.

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*.

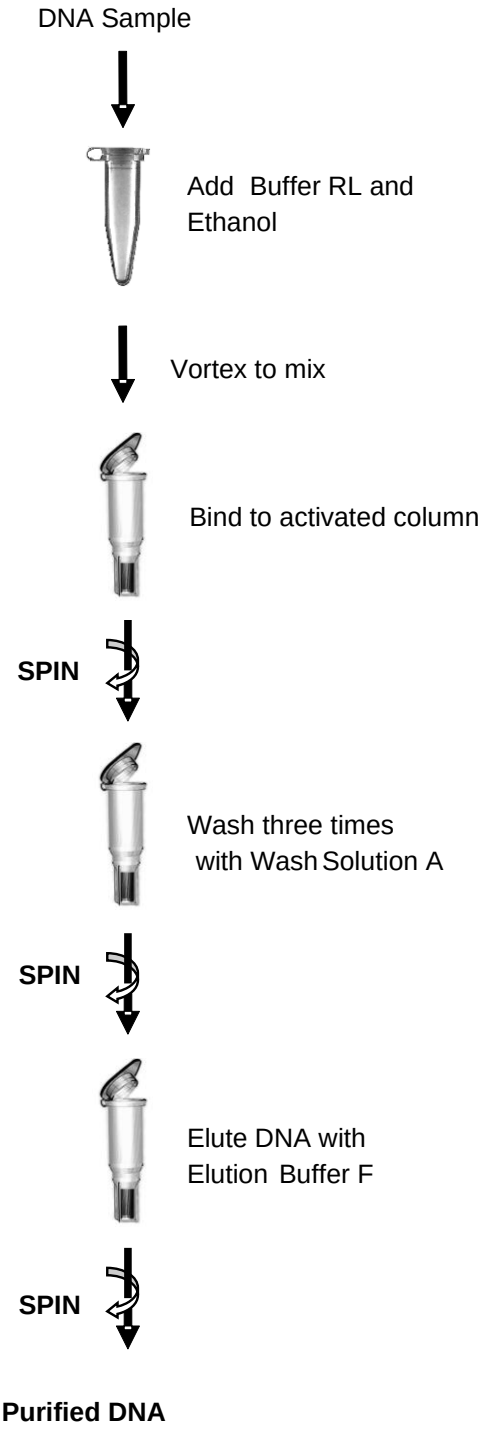
Buffer RL contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment
You must have the following in order to use the DNA Clean-up and Concentration Micro Kit:

- Benchtop microcentrifuge
- 96 - 100% ethanol

Flow Chart

Procedure for Purifying DNA using Norgen's DNA Clean-up and Concentration Micro Kit



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.

Protocol for DNA Clean-Up and Concentration from Enzymatic Reactions or Previously Isolated DNA

All centrifugation steps are carried out in a benchtop microcentrifuge at 20,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

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.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 40 μg of DNA be used per cleanup.
- The maximum volume of DNA sample that can be processed is 200 μL.

1. Sample Preparation

a. Adjust the volume of the DNA sample to 100 μL by adding nuclease free water. It is recommended that no more than 40 μg of DNA be used for each column.

Note: If an input volume between 100 and 200 μL is used, adjust the sample volume to 200 μL (maximum allowable) with nuclease free water. In this case, use the volumes indicated in **bold** in the bracket in Steps **1b** and **1c.**

b. Add 250 μL **(or 500 μL)** of **Buffer RL** to the DNA sample. Mix by vortexing.

c. Add 200 μL **(or 400 μL)** of 96 – 100% ethanol (provided by the user) to the mixture from **Step 1b.** Mix by vortexing for 10 seconds.

2. Column Activation and Sample Binding to Column

a. Assemble a column with one of the provided collection tubes.

b. Apply 500 μL of **Column Activation Solution** onto the column and centrifuge for 1 minute.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.

d. Apply up to 600 μL of the DNA sample with the ethanol (from **Step 1c**) onto the **activated column** and centrifuge for 1 minute.

e. Discard the flowthrough. Reassemble the spin column with its collection tube.

f. If the volume of the DNA sample is greater than 600 μL, repeat **Steps 2d** and **2e** until all the remaining DNA sample has passed through the column.

3. Column Wash

a. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash the column a second time.
d. Wash column a third time by adding another 400 μL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 8 – 15 μL of **Elution Buffer F** to the column.

Note: For maximum concentrations of DNA, the 8 elution μL volume may be used. For maximum recovery of DNA the 15 μL volume is recommended

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **20,000 x *g* (~14,000 RPM).** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 20,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b and 4c**).

5. Storage of DNA

The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution solution was used	It is recommended that the Elution Buffer F supplied with this kit be used for maximum DNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
Clogged Column	High amounts of DNA in the input	Ensure that no more than 40 μg of DNA is used as the input for each column.
	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 columns to clog.
DNA is Degraded	DNase contamination	DNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with DNA.
	Starting size of DNA > 10,000 bp	When purifying large plasmids >10,000 bp in size, DNA may be sheared into smaller fragments.
	Improper storage of the purified DNA	DNA samples may be stored at 4°C for a few days. It is recommended that samples be stored at –20°C for longer term storage.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	DNA was not washed three times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
RNA Clean-Up and Concentration Micro-Elute Kit	61000
PCR Purification Kit	14400, 24800, 45700

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.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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