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Phage DNA Isolation Kit

Product Insert

Product # 46850

Norgen's Phage DNA Isolation Kit provides a rapid method for the isolation and purification of total DNA from bacteriophages propagated in bacteria grown in liquid cultures. The DNA is isolated without the use of phenol, chloroform or cesium chloride. The spin-column based procedure is rapid, and can be completed in less than 45 minutes. The kit is highly efficient for processing small volumes of phage supernatant (1 mL). The purified DNA is of the highest integrity, and can be used in a number of downstream applications including Southern Blot, Restriction Fragment Length Polymorphism (RFLP), sequencing, cloning and real time PCR.

Norgen's Purification Technology

Purification is based on spin column chromatography. The phage DNA is preferentially purified from other cellular components such as proteins without the use of phenol, chloroform or cesium chloride. The starting material for this procedure is clarified phage supernatant that has been separated from bacterial debris in liquid cultures. Initially the phage particles are lysed through a heat and chemical lysis process with the provided Lysis Buffer B (please see the flow chart on page 4). Isopropanol is added to the lysate, and the solution is loaded onto a spin-column. Norgen's spin column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while most of the RNA and proteins are removed in the flowthrough. The bound DNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified total phage DNA is of the highest integrity, and can be used in a number of downstream applications.

Kit Components

Component	Product # 46850 (100 samples)
Lysis Buffer B	2 x 40 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	2 x 8 mL
Spin Columns	100
Collection Tubes	100
Elution tubes (1.7 mL)	100
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Advantages

- Isolate high quality DNA from a variety of phage strains
- High yields of total DNA
- Fast and easy processing using a rapid spin-column format
- No phenol or chloroform extractions or cesium chloride banding required

Specifications

Kit Specifications	
Column Binding Capacity	50 μg
Maximum Column Loading Volume	650 μL
Size of DNA Purified	All sizes
Maximum Amount of Starting Material	1×10^{10} pfu/mL enriched phages
Average Yield*	3-15 μg DNA from 10^6-10^{10} pfu/ mL of enriched phages
Time to Complete 10 Purifications	45 minutes

**Average yields will vary depending upon a number of factors including type of phage, growth conditions used and developmental stage.*

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.

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

You must have the following in order to use the Phage DNA Isolation Kit:

- Benchtop microcenrifuge
- 5mL or 15 mL tube
- 96-100% ethanol
- Isopropanol
- 65°C Heating block or water bath
- Proteinase K - 20 mg/mL (optional)
- DNase I - 2 unit/μL(optional)

Flow Chart

Procedure for Purifying Total DNA using Norgen's Phage DNA Isolation Kit

Phage supernatant prepared from liquid cultures

Add Lysis Buffer B. Incubate.
Add Isopropanol.

Bind to column

SPIN

Wash three times
with Wash Solution A.

SPIN

Elute DNA with
Elution Buffer B.

SPIN

Purified Phage DNA

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

- The starting material for this procedure is clarified phage supernatant. Propagate the phage in liquid cultures and prepare the phage supernatant according to molecular biology manuals or the instructions of the vector supplier.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of **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(s) has a box that may be checked to indicate that the ethanol has been added.
- Preheat a water bath or heating block to 65ºC.
- The maximum amount of starting material is 1 mL of phage supernatant containing 1×10^{10} pfu. For optimal DNA recovery we recommend a minimum phage concentration of at least 1×10^{8} pfu in the 1 mL input.

1. Lysate Preparation

a. The starting material for this procedure is clarified phage supernatant prepared from liquid culture. Transfer 1 mL of the phage lysate (> 1×10^{8} pfu/mL) into a 5 mL or 15 mL tube (provided by the user).

Optional DNase Treatment

If phages are enriched in the cultural media with a host, DNase I treatment is necessary in order to avoid host genomic DNA contamination in the phage DNA elution. This step should be performed at this point in the protocol. Norgen's RNase-Free DNase I Kit (Product #25710) is recommended for this step. Add 10μL (20 units) of Norgen's RNase-Free DNase I and incubate for 15 minutes at room temperature, followed by ***DNase I inactivation at 75°C for 5 minutes.*** Proceed to Step b after the incubation.

b. Add 500 μL of **Lysis Buffer B.** Vortex vigorously for 10 seconds.

Optional Step - Add 4 μL of Proteinase K (20 mg/mL) and incubate at 55°C for 30 minutes. This optional step will help to slightly increase DNA yields.

c. Incubate at 65ºC for 15 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.

d. Add 320 μL of isopropanol (provided by the user) to the lysate. Brief vortex to mix.

2. Sample Binding to Column

a. Assemble a spin column with one of the provided collection tubes. Apply up to 650 μL of the lysate to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).

b. Discard the flowthrough. Reassemble the column and the collection tube.

Note: Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

c. Repeat step **2a and 2b** with remaining lysate until the entire lysate has passed through the column.

3. Column Wash

a. Apply 400 µL of **Wash Solution A** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

b. Wash column a second time by adding 400 µL of **Wash Solution A** and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

c. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

d. Spin the column for 2 minutes in order to thoroughly dry the resin at 14,000 x g (~14,000 RPM). 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 75 µL of **Elution Buffer B** to the column.

c. Centrifuge for 1 minutes at 6,000 x g (~8,000 RPM)

(Optional): A second elution may be performed if desired by repeating steps **4b** and **4c**. It is recommended that the second elution be performed into a separate elution tube. The total yield can be improved by an additional 20-30% when this second elution is performed.

5. Storage of DNA

The purified DNA sample may be stored at –20 °C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The spin column is clogged.	Centrifugation speed was too low or spin time was inadequate.	Check the centrifuge to ensure that it is capable of generating the require RPMs. Sufficient centrifugal force is required to move the liquid through the resin. Also ensure that the correct spin times are followed. Spin for an additional minute if necessary.
	Bacterial debris in the lysate	Ensure that the starting material is clarified phage supernatant. Ensure that bacterial debris from the initial phage supernatant is removed by centrifugation at 10,000 × g for 5 minutes before beginning the protocol.
	The lysate/binding solution mixture is not homogeneous.	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.
The yield of genomic DNA is low	Ineffective propagation of phage and initial lysis step.	Refer to manufacturers recommendations for the propagation of the phage, including proper titer for inoculation, growth conditions, and bacterial host.
	Incomplete lysis of cells	Ensure that the incubation was performed for 15 minutes at 65ºC after the addition of Lysis Buffer B. Also perform optional digestion with Proteinase K in Step 1b during Lysate Preparation.
	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.
	The DNA elution is incomplete	Ensure that all the DNA is eluted. If elution buffer is remained in the column, use 14,000 g for the second centrifuge.
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.

Problem	Possible Cause	Solution and Explanation
Host genomic DNA contamination in phage DNA elution	Phages were enriched in the culture media using a host	In order to eliminate host genomic DNA contamination in the phage DNA elution, it is recommended that a DNAse I treatment is performed at the beginning of the lysate preparation procedure (see Section 1, Optional DNAse Treatment).

Related Products	Product #
Norgen's RNase-Free DNase I Kit	25710
Bacterial Genomic DNA Isolation Kit	17900
Plant/Fungi DNA Isolation kit	26200
Fungi/Yeast Genomic DNA Isolation kit	27300
Soil DNA Isolation Plus Kit	64000, 64060

Technical Support
Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

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