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Biofilm DNA Isolation Kit Product Insert

Product # 62300

Norgen's Biofilm DNA Isolation Kit is designed for the rapid preparation of genomic DNA from biofilm. Genomic DNA is efficiently extracted from the biofilm by a combination of heat treatment, detergents and the use of provided Bead Tubes. The kit is able to remove known PCR inhibitors typically found within the biofilm, including humic acid. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications such as PCR and Southern Blot analysis. Preparation time for a single sample is less than 45 minutes, and each kit contains sufficient materials for 50 preparations.

Norgen's Purification Technology

Purification is based on spin column chromatography. The process involves first adding Lysis Buffer L and Lysis Additive A to the biofilm sample. At this point, an optional RNase treatment can be performed if RNA-free genomic DNA is required. Next, the mixture is incubated at 65˚C and then placed into a provided Bead Tube for a 5 minute homogenization in order to efficiently and rapidly break the biofilm and bacteria to extract the DNA. The sample is then centrifuged, and the supernatant is transferred to a DNAse-free microcentrifuge tube. Ethanol is added to the lysate, and it is then loaded onto one of the provided spin columns. Norgen's columns bind nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins are removed in the flowthrough or retained on top of the column. The bound DNA is then washed twice using the provided Wash Solution A, and the purified DNA is eluted using the Elution Buffer. The purified DNA can be used in sensitive downstream applications including PCR and Southern blotting.

Kit Specification

Kit Specifications	
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Minimum Biofilm Input (wet weight)	50 mg
Maximum Biofilm Input (wet weight)	200 mg
Average Yield from 200 mg of biofilm *	2-4 µg
Time to Complete 10 Purifications	45 minutes

* Yield will vary depending on the type of biofilm sample processed

Advantages

- Rapid and convenient method to isolate genomic DNA from different types of biofilm and biofilm forming-bacteria
- No phenol or chloroform extractions
- Yields high quality DNA that is ready for PCR and other downstream applications.

Kit Components

Component	Product # 62300 (50 preps)
Lysis Buffer L	30 mL
Lysis Additive A	6 mL
Binding Buffer I	7 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Bead Tubes	50
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. These reagents should remain stable for at least 2 years 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*.

Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 65°C water bath or heating block
- 96 – 100% ethanol
- Homogenizer or motor and pestle
- RNase A (optional)

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:

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

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 precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat a water bath or heating block to 65°C.

1A. Lysate Preparation for Biofilm Species.

a. Transfer 50-200mg of biofilm (wet weight) to a microcentrifuge tube.
b. Centrifuge at 20,000 x g (~14,000 RPM) for 1 minute to collect the biofilm. Discard the liquid carefully.
c. Transfer carefully the biofilm to a Bead Tube (provided).
d. Add 500 µL of **Lysis Buffer L** to the bead tube. Follow with gentle vortexing for 10 seconds.
e. Add 100 µL of **Lysis Additive A**. Vortex to mix.

   Optional: At this point, an optional RNase treatment can be performed if RNA-free genomic DNA is required. Add 1 µL of 10 µg/mL RNase A to the lysate and briefly vortex to mix. Proceed Step 1A f.

f. Incubate the Bead Tube with lysate at 65°C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
g. To homogenize, vortex the tube horizontally on a flat-bed vortex pad with tape, or in any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep® -24 or Scientific Industries' Disruptor Genie^{TM}). Vortex for 4M/S for 30 seconds for MP Biomedicals' FastPrep® -24 or for 5 minutes for a flat-bed vortex. Optimize the condition for any commercially available bead beater equipment

   Note: Foaming during the homogenization is common. This foaming is due to detergents present in the Lysis Buffer L and will not affect the protocol.

i. Spin the bead tubes at **20,000 × g (~14,000 RPM) for 1 minute** and carefully transfer all of the lysate to a DNase-free microcentrifuge tube (provided by the user) by pipetting. **Ensure that the beads and debris are not transferred during pipetting.**
j. Add 100 µL of **Binding Buffer I** to the clear supernatant. Vortex briefly.
k. Incubate at on ice for 5 minutes.
l. Centrifuge at **20,000 × g (~14,000 RPM) for 3 minutes**.
m. Carefully transfer clean supernatant to a new DNase-free microcentrifuge tube (provided by the user) without disturbing the pellet. Note the volume.
n. Add an equal volume of 70% ethanol (not provided) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2: Binding to Column**

2. Binding DNA to Column

a. Obtain a spin column assembled with its collection tube.
b. Apply up to 650 µL of the lysate with ethanol onto the column and centrifuge for 1 minute at **10,000 x g (~10,000 RPM)**. Discard the flowthrough and reassemble the spin column with the collection tube.

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

c. Repeat step **2b** with remained lysate.

3. Column Wash

a. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute at **10,000 xg (~10,000 RPM)**.

Note: Ensure the entire wash solution 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 column with its collection tube.
c. Repeat steps **3a and 3b** to wash column a second time.
d. Spin the column for 2 minutes at **20,000 x *g* (~14,000 RPM)** in order to thoroughly dry the column. 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 100 μL of **Elution Buffer B** to the column.
c. Incubate at room temperature for 1 minute.
d. Centrifuge for 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 14,000 x g (~14,000 RPM) for 1 additional minute.

Optional: An additional elution may be performed if desired by repeating steps **4b** to **4d** using 100 μL of **Elution Buffer B**. To prevent dilution of the elution it is recommended that a separate elution tube is used for the second elution. The total yield can be improved by an additional 20 – 30% when this second elution is performed.

5. Storage of DNA

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

Related Products	Product #
HighRanger 1kb DNA Ladder	11900
Fungi/Yeast Genomic DNA Isolation Kit	27300
Bacterial Genomic DNA Isolation Kit	17900
Plant/Fungi DNA Isolation Kit	26200

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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The spin column is clogged.	The sample is too large	Too much biofilm was applied. Ensure that the amount of sample used is between 50 – 200 mg (wet weight). Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
Poor DNA Recovery	Lysis was not completed	Increase the incubation time at 65ºC to 15-20 minutes.
	Ethanol was not added to the Wash Solution A	Ensure that 42 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	70% Ethanol was not added to the lysate	70% ethanol enhances DNA binding to the column for maximum DNA recovery.
DNA does not perform well in downstream applications	The sample is too large	Too much biofilm was applied. Ensure that the amount of sample used does not exceed 200mg (wet weight).
	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.
	The column was not washed twice with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed twice with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	PCR reaction conditions need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template (20 ng to 50 ng for 20 µL of PCR reaction), changing the source of *Taq* polymerase, adding BSA (final concentration is 0.1 µg/µl), looking into the primer design and adjusting the annealing conditions.

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