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Bacterial Genomic DNA Isolation 96-Well Kit Product Insert

Product # 17950

Norgen's **Bacterial Genomic DNA 96-Well Kit** provides a rapid method for the high-throughput isolation of genomic DNA from 2 x 10^9 viable bacterial cells (between 0.5 and 1.0 mL of culture). Purification is based on 96-well column chromatography as the separation matrix. Norgen's 96-well plate binds DNA under optimized salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including real-time PCR and Southern Blot analysis.

Norgen's Bacterial Genomic DNA Isolation 96-Well Kit allows for the isolation of genomic DNA from both Gram-negative and Gram-positive cultures, including *Escherichia coli* and *Bacillus cereus*. The genomic DNA is preferentially purified from other cellular proteinaceous components. Typical yields of genomic DNA will vary depending on the cell density of the bacterial culture and the bacterial species. Preparation time for a single 96-well plate is less than 90 minutes, and each kit contains sufficient materials for 192 preparations.

Kit Components

Component	Product #17950 (192 preps)
Resuspension Solution A	60 mL
Lysis Buffer P	60 mL
Solution BX	110 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	2 x 30 mL
Proteinase K	50 mg
96-Well Plate	2
Adhesive Tape	4
Collection Plate	2
Elution Plate	2
Product Insert	1

Specifications

Kit Specifications	
Maximum Input	2 x 10^9 bacterial cell
Average Yield	Up to 20 µg*
Well Binding Capacity	25 µg
Time to Complete 96 Purifications	90 minutes

* Yield will vary depending on the type of sample processed

Advantages

- Fast and easy processing using either a vacuum manifold or centrifugation
- Isolate high quality genomic DNA
- Isolate genomic DNA from various inputs including both Gram-negative and Gram-positive cultures, including *Escherichia coli* and *Bacillus cereus*
- Recovered genomic DNA is compatible with various downstream applications

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. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 1 year after the date of shipment when stored at room temperature.

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 **Solution BX** 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

You must have the following in order to use the Bacterial Genomic DNA Isolation 96-Well Kit:

- 96 - 100% ethanol
- Collection/Waste Tray for vacuum manifold. Two 96-Well Collection Plates are provided with the kit.
- 55°C water bath or incubator.
- 37ºC water bath or incubator (for Gram-positive strains only)
- RNase A (optional)
- Lysozyme (for Gram-positive strains only)
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
    - Sealing tape or pads
- For ***Centrifuge Format***:
    - Centrifuge with rotor for 96-well plate assembly, such as AllSpin Js-5.3 Rotor for Avanti® J-26xp centrifuge, Beckman Coulter or similar rotor that can hold the stack of the 96-well Plate and the Collection Plate and that can reach the minimum speed of 4000 rpm (~4000xg)

Flow Chart

Procedure for Purifying Bacterial Genomic DNA using Norgen's Bacterial Genomic DNA Isolation 96-Well Kit

Transfer bacterial culture to a microcentrifuge tube

SPIN

Resuspend cell pellet in Resuspension Solution A. Add Lysis Buffer P and Proteinase K (For Gram Positive Bacteria: add Lysozyme)

Incubate for 30 minutes at 55°C (For Gram Positive Bacteria incubate for 2 hours at 37°C).
Add Solution BX and ethanol

Bind

Wash twice with Wash Solution A.
Dry plate

Elute DNA with Elution Buffer B

Pure Bacterial Genomic DNA

Procedure

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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 precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Lysate preparation is different for Gram-negative and Gram-positive bacteria. For Gram-negative bacteria, please follow the procedure outlined in Section 1A. For Gram-positive bacteria, please follow the procedure outlined in Section 1B.
- The isolation of Bacterial Genomic DNA can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of **96-100% ethanol** (provided by user) to the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Reconstitute the **Proteinase K** in 2.5 mL of molecular biology grade water, aliquot in 120 µL fractions (or larger fractions) and store the unused portions at -20°C until needed.
- The input bacterial cell amount should not exceed 2 x 10^9 cfu's. Depending on culture growth, this is equivalent to 0.5 -1.0 mL of an overnight culture. It is not recommended to exceed 1 mL of culture for this procedure.
- Preheat a water bath or heating block to 55°C (37ºC for Gram-positive strains).
- **For Gram-positive bacteria**, prepare a 400 mg/mL stock solution (approximately 1.7 x 10^7 units/mL) of lysozyme (provided by user) as per supplier's instructions.
- For the optional RNase treatment step, RNase (provided by user) of a minimum concentration of 10 KUnitz per 20 µL should be used.

1. Lysate Preparation

1A. Lysate Preparation (Gram-Negative Bacteria)

a. Transfer up to 1 mL of bacterial culture to a microcentrifuge tube and centrifuge at 14,000 x g (~14,000 RPM) for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 250 µL of **Resuspension Solution A** to the cell pellet. Resuspend the cells by gentle vortexing.

Optional RNase A treatment: If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step **1c**.

c. Add 250 µL of the **Lysis Buffer P** and 12 µL of **Proteinase K** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 30 minutes.

Note: Incubation times may fluctuate between 15 and 45 minutes depending on the amount and type of bacterial strain being lysed. Lysis is considered complete when a relatively clear lysate is obtained. Slight cloudiness in the lysate may persist for certain strains, which will not affect the genomic DNA recovery.

d. Add 500 µL of **Solution BX** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
e. Add 500 µL of 96-100% ethanol (provided by user) and mix well with gentle vortexing.
f. Proceed to Step 2: Binding to Plate.

1B. Lysate Preparation (Gram-Positive Bacteria)

a. Transfer up to 1 mL of bacterial culture to a microcentrifuge tube and centrifuge at 14,000 *x g* (~14,000 RPM) for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.
b. Add 250 µL of **Resuspension Solution A** to the cell pellet. Resuspend the cells by gentle vortexing.
c. Add 12 µL of previously prepared lysozyme stock solution and mix well**.**

Optional RNase A treatment: If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step **1d**.

d. Add 250 µL of the **Lysis Buffer P** and 12 µL of **Proteinase K** to the cell suspension. Mix well by gentle vortexing and incubate at 37°C for 2 hours.

Note: Incubation times may fluctuate between 0.5 and 2 hours depending on the bacterial strain being lysed. Lysis is considered complete when a relatively clear lysate is obtained. Slight cloudiness in the lysate may persist for certain strains, which will not affect the genomic DNA recovery.

e. Add 500 µL of **Solution BX** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
f. Add 500 µL of 96-100% ethanol (provided by user) and mix well with gentle vortexing.
g. Proceed to Step 2: Binding to Plate.

2. Genomic DNA Isolation

Note: The purification of Genomic DNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in 2A. For purification using centrifugation, please follow the procedure outlined in 2B

A. Genomic DNA isolation Using Vacuum Manifold

1. Binding DNA to 96-Well Plate

a. Assemble the 96-Well Plate and the vacuum manifold according to manufacturer's recommendations.
b. Apply 750 µL of the lysate into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

d. Apply the rest of the lysate into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
e. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

2. DNA Wash

a. Apply 500 µL of the **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution A has passed through into the collection/waste tray by inspecting the 96-Well Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Plate and the vacuum manifold.
d. Apply another 500 µL of the **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells and apply vacuum for 2 minutes.
e. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Pat the bottom of the 96-Well Plate gently to remove any residual wash buffer. Reassemble the 96-Well Plate and the vacuum manifold.
f. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
g. Turn off vacuum and ventilate the manifold. Ensure that the bottom of the plate is dry, and blot any excess moisture onto a paper towel if necessary.

3. DNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
b. Add 200 µL of **Elution Buffer B** to each well of the plate.
c. Apply vacuum for 3 minutes.

Optional: The yield can be improved by an additional 20-30% by pipetting the elution back onto the plate and repeating Step 3c.

4. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Genomic DNA Purification Using Centrifugation

Note: To purify genomic DNA using a vacuum manifold please follow Section A above.

1. Binding DNA to 96-Well Plate

a. Place the 96-Well Plate on top of the Collection Plate.

Note: The user should ensure that the assembled 96-Well Plate and the Collection Plate stack fits into the rotor without interfering with the centrifugation process.

b. Apply 750 µL of the lysate into each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 3 minutes.
c. Discard the flowthrough. Reassemble the 96-Well Plate and the Collection Plate.

Note: Ensure that all of the lysate from each well has passed through into the Collection Plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

d. Apply the rest of the lysate into each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 3 minutes.
e. Discard the flowthrough. Reassemble the 96-Well Plate and the Collection Plate.

2. DNA Wash

a. Apply 500 µL of the **Wash Solution A** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the Collection Plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the Collection Plate.
c. Apply another 500 µL of the **Wash Solution A** to each well of the 96-Well Plate.
d. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 2 minutes.
e. Pat the bottom of the 96-Well Plate dry. Reassemble the 96-Well Plate and the Collection Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 15 minutes in order to completely dry the plate. Ensure that the bottom of the plate is dry, and blot any excess moisture onto a paper towel if necessary.

3. DNA Elution

a. Stack the 96-Well Plate on top of the Elution Plate.
b. Add 200 µL of **Elution Buffer B** to each well of the 96-Well Plate.
c. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 3 minutes.

Optional: The yield can be improved by an additional 20-30% by pipetting the elution back onto the plate and repeating Step 3c.

4. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples 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 96 well plate is clogged.	The sample is too large	Too many cells were applied to the well. Ensure that the amount of cells used is less than 2 x 10^9 viable cells, and that no more than 1 mL of culture is applied to the well. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the well.
	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	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 wells to clog.
The lysate is very gelatinous prior to loading onto the wells	The lysate/ Solution BX mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the well.
	The sample is too large	Too many cells are in the lysate preparation. Ensure that the amount of cells used is less than of 2 x 10^9 viable cells, and that no more than 1 mL of culture is applied to the well.
The yield of genomic DNA is low	The sample is old/overgrown	The culture may have been overgrown, allowing lysis of older cells to occur more readily. This will lead to premature degredation of the genomic DNA. It may be necessary to use bacterial cultures before they reach maximum density.
	Incomplete lysis of cells	Extend the incubation time of Proteinase K digestion or reduce the amount of bacterial cells used for lysis. Increase the lysozyme incubation time for Gram-positive strains.
The genomic DNA is sheared	The genomic DNA was handled improperly	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).
	The cells are old	Older cultures contain prematurely lysed cells which release endonucleases and can degrade DNA. Fresh cultures are recommended.

DNA does not perform well in downstream applications.	DNA was not washed two times with the provided Wash Solution A	Ensure the plate was washed two times with Wash Solution A. An additional wash with Wash Solution A can improve DNA performance in downstream applications, however it may reduce DNA yield.
	Ethanol carryover	Ensure that the plate dry step under the 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 #**
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100
Milk Bacterial DNA Isolation Kit	21500

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.

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