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Bacterial Genomic DNA Isolation Kit — Product Insert

Product # 17900

Norgen's **Bacterial Genomic DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from 2 x 10^9 viable bacterial cells (between 0.5 and 1.0 mL of culture). Purification is based on spin column chromatography as the separation matrix. Norgen's column binds DNA under high 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 PCR and Southern Blot analysis.

The Bacterial Genomic DNA Isolation 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 sample is approximately 45 minutes, and each kit contains sufficient materials for 50 preparations.

Kit Components

Component	Product # 17900 (50
Resuspension Solution A	20 mL
Lysis Buffer P	18 mL
Solution BX	28 mL
Wash Solution A	18 mL
Elution Buffer B	30 mL
Proteinase K	12 mg
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

The Proteinase K should be stored at -20°C upon arrival and after reconstitution. All other 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*.

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

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 55°C water bath or heating block
- 37°C water bath or heating block (for Gram positive strains only)
- 96 – 100% ethanol
- RNase A (optional)
- Lysozyme (for Gram positive strains only)

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:

- 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.
- Reconstitute the Proteinase K in 0.6 mL of molecular biology grade water, aliquot in 120 µL fractions and store the unused portions at -20°C until needed.
- Add 42 mL of 96-100% ethanol to **Wash Solution A**.
- 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 (37°C 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 as per supplier's instructions.

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. Proceed to Step 2: Binding to Column.

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. Proceed to Step 2: Binding to Column.

2. Binding to Column

a. Add 500 μL of **Solution BX** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.

b. Add 500 μL of 96-100% ethanol (provided by user) and mix well with gentle vortexing.

c. Assemble a spin column with one of the provided collection tubes. Apply 750 μL of the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 1 minute at 5,200 *x g* (~ 8,000 RPM).

d. After centrifugation, discard the flowthrough, and reassemble the spin column with its collection tube.

e. Apply the rest of the mixture to the column, cap the column, and centrifuge the unit for 1 minute at 5,200 *x g* (~ 8,000 RPM).

f. After centrifugation, discard the flowthrough, and reassemble the spin column with its collection tube.

3. Washing Bound DNA

a. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

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. Wash column a second time by adding another 500 µL of **Wash Solution A** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

c. Spin the column for 2 minutes in order to thoroughly dry the column. Discard the collection tube.

4. Elution of Clean DNA

a. Assemble the spin column (with DNA bound to the column) with a provided 1.7 mL **Elution tube.**

b. Add 200 µL of **Elution Buffer B** to the center of the column bed. Centrifuge for 1 minute at **3,000 *x g* (~6,000 RPM)**. A portion of the **Elution Buffer B** will pass through the column which allows for hydration of the DNA to occur.

c. Centrifuge at **14,000 *x g* (~14,000 RPM)** for an additional 2 minutes to collect the total elution volume.

Optional: The yield can be improved by an additional 20-30% by pipetting the elution back onto the column and repeating Step 4c.

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Related Products	Product #
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100

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 many cells were applied to the column. 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 column. 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.
The lysate is very gelatinous prior to loading onto the column	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.
	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 column.
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 DNA elution is incomplete	Ensure that centrifugation at 14,000 *x g* is performed after the 3,000 *x g* centrifugation cycle, to ensure that all the DNA is eluted.
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.

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