

Bacterial Genomic DNA Isolation Kit

Product # 17900

Product Insert

Norgen's **Bacterial Genomic DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from 2×10^9 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 samples) |
|-------------------------|------------------------------|
| 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

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years 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 2 years 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.norgenbiotech.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 μ L 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 2×10^9 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 1.7×10^7 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 20,000 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 μ L 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 **1Ac**.

- 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 20,000 $\times g$ (~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 (400 mg/mL) 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 **1Bd**.

- 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 650 μL of the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 1 minute at 10,000 $\times g$ (~ 10,000 RPM).

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 at 20,000 $\times g$ (~14,000 RPM) for 1 minute.

- 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 10,000 $\times g$ (~ 10,000 RPM).

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 at 20,000 x g (~14,000 RPM) for 1 minute.

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

3. Washing Bound DNA

- a. Apply 500 μ L of **Wash Solution A** to the column and centrifuge for 1 minute at 20,000 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 μ L of **Wash Solution A** and centrifuging for 1 minute at 20,000 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 μ L 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 **20,000 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 2×10^9 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 2×10^9 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 degradation 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 $20,000 \times g$ is performed after the $3,000 \times g$ 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. |

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
 Phone: (905) 227-8848
 Fax: (905) 227-1061
 Toll Free in North America: 1-866-667-4362