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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 using Norgen's proprietary resin as the separation matrix. Norgen's resin 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	18 mL
Lysis Solution	18 mL
Binding Solution	4 mL
Wash Solution I	7.5 mL
Wash Solution II	22.5 mL
Elution Buffer	24 mL
Proteinase K	12 mg
Micro 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 1 year in their unopened containers. The Proteinase K should be stored in aliquots at -20°C upon reconstitution. This product is stable at room temperature in its lyophilized form.

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 **Binding Solution**, **Wash Solution I** and **Wash Solution II** contain 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 ^{o}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 22.5 mL of 96-100% ethanol to **Wash Solution I**.
- Add 7.5 mL of 96-100% ethanol to **Wash Solution II.**

- 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** 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 Solution** 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** 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 Solution** 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 50 µL of **Binding Solution** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
- **b.** Assemble a micro spin column with a provided collection tube. Apply the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 4 minutes at 5,200 *x g* (~ 8,000 RPM).
- **c.** 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 I** to the column, and centrifuge the unit for 1 minute at 14,000 *x g* (~14,000 RPM).
- **b.** After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
- **c.** Apply 500 µL of **Wash Solution II** to the column, and centrifuge the unit for 2 minutes at 14,000 *x g* (~14,000 RPM).
- **d.** Carefully detach the spin column from the collection tube and discard the collection tube and flowthrough.

  Note: If any liquid is left on the side of the spin column, discard the flowthrough and reassemble the spin column with its collection tube. Spin for an additional 1 minute at 14,000 *x g* (~14,000 RPM) in order to completely dry the column.

4. Elution of Clean DNA

- **a.** Assemble the spin column (with DNA bound to the resin) with a provided 1.7 mL **Elution tube.**
- **b.** Add 200 µL of **Elution Buffer** to the center of the resin bed. Centrifuge for 1 minute at **3,000 *x g* (~6,000 RPM)**. A portion of the **Elution Buffer** 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.
- **d.** **(Optional):** An additional elution may be performed if desired. Another 200 µL of **Elution Buffer** may be added to the column and centrifuged at 3,000 *x g* for 1 minute into a new elution tube. Then, centrifuge the column at 14,000 *x g* for an additional 2 minutes. The yield can be improved by an additional 20-30% when this second elution is performed.

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

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The micro 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 of 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 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.

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.

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