Geneaid

Instruction Manual
Ver. 05.25.17
For Research Use Only

Presto™ gDNA Bacteria Advanced Kit

GBBA004 (4 Preparation Sample Kit)
GBBA100 (100 Preparation Kit)
GBBA300 (300 Preparation Kit)

Advantages

Sample: up to 1 x 10^9 Gram (+) positive and Gram (-) negative bacterial cells
gDNA Yield: up to 40 µg from 1 x 10^9 *Escherichia coli* and up to 15 µg from 1 x 10^9 *Bacillus subtilis*
Convenient: includes beadbeating tubes for effective cell lysis
Format: genomic DNA spin columns (sterilised to remove bacteria contamination)
Time: within 30 minutes
Elution Volume: 30-200 µl
Kit Storage: dry at room temperature (15-25°C)

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Presto™ gDNA Bacteria Advanced Kit is designed for rapid isolation of genomic DNA from cultured Gram (+) positive and Gram (-) negative bacteria. After directly adding into pre-filled beadbeating tubes containing zirconia/ceramic beads and lysis buffer, bacterial cells are efficiently lysed using a standard vortex or bead beating instrument. Enzymatic digestion with Lysozyme and Proteinase K is not required. The cell lysate is then mixed with a binding buffer, followed by bind, wash and elute using a spin column designed specifically for bacteria DNA extraction. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 30 minutes. The purified genomic DNA is ready for use in PCR, restriction enzyme digestion, and sequencing reactions.

Quality Control

The quality of the Presto™ gDNA Bacteria Advanced Kit is tested on a lot-to-lot basis by isolating genomic DNA from 1 x10^9 *E. coli* cells. Following the purification process, a yield of more than 30 µg of genomic DNA is obtained and the A260/A280 ratio is between1.7-2.0. The purified genomic DNA is analyzed by electrophoresis.

Kit Components

Component	GBBA004	GBBA100	GBBA300
GT Buffer	1.5 ml x 2	75 ml	200 ml
PR Buffer	1 ml	15 ml	40 ml
GB Buffer	2 ml	60 ml	155 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
RNase A (50 mg/ml)[2]	25 µl	550 µl	550 µl x 3
GD Columns	4	100	300
Beadbeating Tubes Type A	4	100	300
2 ml Collection Tubes	4	100	300

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

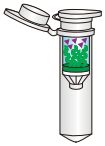
[2]RNase A is shipped at room temperature but should be stored at 4ºC for extended periods.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

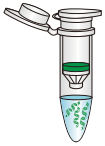
Lysis of bacteria cells using beadbeating tubes and a standard vortex or bead beating instrument.

Enzymatic cell lysis using Lysozyme and Proteinase K is not required.



DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

Presto™ gDNA Bacteria Advanced Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. RNase A is shipped at room temerature but should be stored at 4ºC for extended periods.

Additional Requirements

1.5 ml microcentrifuge tubes, standard vortex or bead beating instrument, absolute ethanol

Protocol Procedure

1. Sample Preparation and Lysis

Transfer **20-100 mg of bacteria cell pellet** (wet weight, up to 1 x10^9 bacteria cells) to a microcentrifuge tube.

NOTE: Transfer liquid bacteria culture to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. Weigh the pellet, repeat to harvest bacterial cells by centrifugation using the same microcentrifuge tube if required.

Add **600 µl of GT Buffer** then re-suspend the cell pellet by vortex or pipette. Transfer the re-suspended bacterial cells and **5 µl of RNase A** to a **Beadbeating Tube**. Attach the Beadbeating Tubes horizontally to a standard vortex with tape or use an adapter. Vortex the Beadbeating Tubes at maximum speed at room temperature for 10 minutes. Carefully open the cap, add **100 µl of PR Buffer** and mix by vortex briefly to eliminate the foam caused by detergents. Incubate the tubes on ice for 5 minutes then centrifuge at 11,000 x g for 3 minutes at room temperature. Transfer **450 µl of supernatant** to a clean 1.5 ml microcentrifuge tube.

NOTE: Preheat required Elution Buffer (200 µl per sample) to 60ºC for step 4 DNA Elution.

2. DNA Binding

Add **450 µl of GB Buffer** and **450 µl of absolute ethanol** to the sample and mix IMMEDIATELY by shaking vigorously for 10 seconds.

NOTE: Equal volumes of GB Buffer and absolute ethanol can be mixed in advance then stored at room temperature. Transfer 900 µl of GB Buffer and ethanol mixture to the sample then mix by shaking vigorously.

Place a **GD Column in a 2 ml Collection Tube**. Transfer **700 µl of sample mixture** to the **GD Column** then centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Transfer the remaining sample mixture to the GD Column then centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.

3. Wash

Add **400 µl of W1 Buffer to the GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure ethanol was added) to the GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 16,000 x g to dry the column matrix.

4. Elution

Transfer the dry **GD Column** to a new 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 200 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Incomplete sample lysis or homogenization.

Horizontally vortex the Beadbeating Tube at maximum speed using a vortexer at room temperature for 10 minutes or use a Disruptor Genie or similar.

Incorrect DNA elution.

Pre-heat the Elution Buffer to 60ºC prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely.

Incomplete buffer preparation.

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Degraded DNA

Mechanical sample disruption is too vigorous.

Alternative lysis method for less DNA shearing: After adding bacterial cells and GT Buffer, vortex the Beadbeating Tube at maximum speed for 5 seconds then incubate the Beadbeating Tube at 70ºC for 5 minutes. Repeat these steps 3 times. This lysis method will reduce DNA shearing but may also reduce DNA yield.

DNA Contaminated RNA

RNA carry-over.

Add 5 µl of RNase A (50 mg/ml) to the sample before cell lysis by vortex.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

Presto™ gDNA Bacteria Advanced Kit Test Data

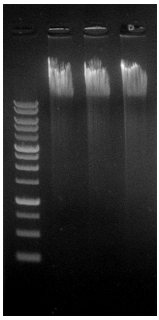


Figure 1. Genomic DNA was extracted using the Presto™ gDNA Bacteria Advanced Kit from 50 mg of *Escherichia coli* pellet. A 2 µl aliquot of purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Sample	ng/µl	260/280	260/230	Yield (µg)
1	312.1	1.89	2.34	31.2
2	341.6	1.88	2.35	34.2
3	332.8	1.88	2.32	33.3

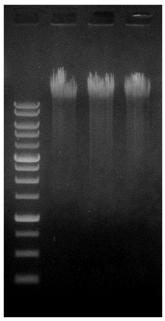


Figure 2. Genomic DNA was extracted using the Presto™ gDNA Bacteria Advanced Kit from 50 mg of *Bacillus subtilis* pellet. A 2 µl aliquot of purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Sample	ng/µl	260/280	260/230	Yield (µg)
1	121.8	1.91	2.39	12.2
2	130.9	1.91	2.40	13.1
3	133.6	1.89	2.39	13.4

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