Geneaid

Instruction Manual
Ver. 02.10.17
For Research Use Only

Presto™ Mini gDNA Bacteria Kit

GBB004 (4 Preparation Sample Kit)
GBB100/101 (100 Preparation Kit)
GBB300/301 (300 Preparation Kit)

Advantages

Sample: up to 1 x 10^9 Gram (+) positive and Gram (-) negative bacterial cells, 200 µl of blood and biological fluids such as plasma, urine etc.
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 Gram+ Buffer for preparing lysozyme solutions and to speed up sample preparation
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), Lysozyme is shipped at room temperature and should be stored at -20°C for extended periods

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Introduction

The Presto™ Mini gDNA Bacteria Kit is optimized for genomic and viral DNA purification from Gram (-) negative and Gram (+) positive bacterial cells, whole blood and biological fluids. Gram+ Buffer, when combined with Lysozyme, will efficiently lyse bacterial cell walls consisting of the peptidoglycan layer. Proteinase K and chaotropic salt are used to further lyse cells and degrade protein, allowing DNA to easily bind to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. Phenol/chloroform extraction or alcohol precipitation is not required and the purified genomic DNA is ready for use in a variety of downstream applications.

Quality Control

The quality of the Presto™ Mini gDNA Bacteria Kit is tested on a lot-to-lot basis by isolating DNA from *Escherichia coli* (1×10^9) culture harvested by centrifugation at 16,000 x g for 1 minute. 5 µl from a 100 µl eluate of purified DNA is analyzed by electrophoresis on a 1% agarose gel.

Kit Components

Component	GBB004	GBB100	GBB101	GBB300	GBB301
Gram+ Buffer	2 ml	30 ml	30 ml	75 ml	75 ml
GT Buffer	1.5 ml	30 ml	30 ml	75 ml	75 ml
GB Buffer	2 ml	40 ml	40 ml	75 ml	75 ml
W1 Buffer	2 ml	45 ml	45 ml	130 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)	50 ml (200 ml)	50 ml (200 ml)
Lysozyme[2]	8 mg	110 mg	N/A	250 mg	N/A
Proteinase K[3] (Add ddH_2O)	1 mg (0.1 ml)	11 mg x 2 (1.1 ml)	11 mg x 2 (1.1 ml)	65 mg (6.5 ml)	65 mg (6.5 ml)
Elution Buffer	1 ml	30 ml	30 ml	75 ml	75 ml
GD Columns	4	100	100	300	300
2 ml Collection Tubes	8	200	200	600	600

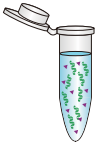
[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]Lysozyme should be stored at -20ºC for extended periods.

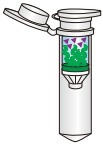
[3]Add ddH_2O (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

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

Quick Protocol Diagram

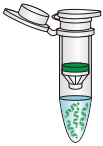


Sample preparation and cell lysis of bacteria, whole blood and biological fluids



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™ Mini gDNA Bacteria 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. Add ddH_2O (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

Additional Requirements

absolute ethanol, microcentrifuge tubes, pipette tips, RNase A (50 mg/ml), 15 ml centrifuge tube (Gram positive bacteria only)

Protocol Procedure

1. Sample Preparation

Gram (-) Negative Bacteria

Transfer **bacterial cells (up to 1 x 10^9)** to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. Add **180 µl of GT Buffer** then re-suspend the cell pellet by vortex or pipette. Add **20 µl of Proteinase K (make sure ddH_2O was added)**. Incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes. Proceed with step 2 Lysis.

Gram (+) Positive Bacteria

Transfer **bacterial cells (up to 1 x 10^9)** to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. **Transfer the required volume of Gram+ Buffer (200 µl/sample)** to a 15 ml centrifuge tube. Add **Lysozyme (0.8 mg/200 µl) to Gram+ Buffer (in the 15 ml centrifuge tube)** then vortex to completely dissolve the Lysozyme. Transfer **200 µl of Gram+ Buffer (make sure Lysozyme was added)** to the sample in the 1.5 ml microcentrifuge tube then re-suspend the pellet by vortex or pipette. Incubate at 37ºC for 30 minutes. During incubation, invert the tube every 10 minutes. Add **20 µl of Proteinase K (make sure ddH_2O was added)** then mix by vortex. Incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes. Proceed with step 2 Lysis.

Whole Blood

Transfer **200 µl of blood** to a 1.5 ml microcentrifuge tube. Add **20 µl of Proteinase K (make sure ddH_2O was added)** then mix by vortex for 10 seconds. Incubate at 60ºC for at least 10 minutes. During incubation invert the tube every 3 minutes. Proceed with step 2 Lysis.

Biological Fluids

Transfer **1 ml of biological fluid** to a 1.5 ml microcentrifuge tube. Centrifuge for 5 minutes at 14-16,000 x g then discard the supernatant.

NOTE: When using more than 1 ml of biological fluid, repeat the centrifuge step.

Add **200 µl of GT Buffer** then re-suspend the pellet by vortex or pipette. Add **20 µl of Proteinase K (make sure ddH_2O was added)** then mix by vortex. Incubate at 60ºC for at least 10 minutes. During incubation invert the tube every 3 minutes. Proceed with step 2 Lysis.

2. Lysis

Add **200 µl of GB Buffer** to the sample and mix by vortex for 10 seconds. Incubate at 70ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, pre-heat the required Elution Buffer (200 µl per sample) to 70ºC (for step 5 DNA Elution).

Optional RNA Removal Step

Following 70ºC incubation, add 5 µl of RNase A (50 mg/ml) to the clear lysate then shake vigorously. Incubate at room temperature for 5 minutes.

3. DNA Binding

Add **200 µl of absolute ethanol** to the sample lysate and mix **IMMEDIATELY** by shaking vigorously. If precipitate appears, break it up as much as possible with a pipette. Place a **GD Column** in a 2 ml Collection Tube. **Transfer mixture (including any insoluble precipitate) to the GD Column** then centrifuge at 14-16,000 x g for 2 minutes. Discard the 2 ml Collection Tube containing the flow-through then place the **GD Column** in a new 2 ml Collection Tube.

4. Wash

Add **400 µl of W1 Buffer to the GD Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. 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 14-16,000 x g for 30 seconds then discard the flow-through. Place the **GD Column** back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

5. Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GD Column** to a clean 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 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) 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 buffer preparation.

1. When extracting genomic DNA from Gram (+) positive bacteria, add Lysozyme to Gram+ Buffer immediately prior to use.

2. 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.

3. Add ddH_2O (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

Incomplete cell lysis.

Reduce the amount of starting material or separate into multiple tubes. Make sure bacteria cells were completely homogenized in GT Buffer or Gram+ Buffer. If extracting genomic DNA from Gram (+) positive bacteria, make sure Lysozyme is added to Gram+ Buffer prior to use.

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GD Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70ºC). 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. Repeating the elution step will increase yield. Repeating the elution step using the eluate only will increase DNA concentration.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

Presto™ Mini gDNA Bacteria Kit Functional Test Data

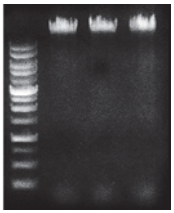


Figure 1. Genomic DNA (approximately 30 kb) was extracted using the Presto™ Mini gDNA Bacteria Kit. An *Escherichia coli* (1×10^9) culture (OD600=2, 1 ml) was harvested by centrifugation at 16,000 x g for 1 minute. A 5 µl aliquot of purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel. M = Geneaid 1 Kb DNA Ladder

Test	DNA Yield	260/280	260/230
1	38.16 µg	2.05	2.26
2	33.88 µg	2.05	2.27
3	39.02 µg	2.07	2.29

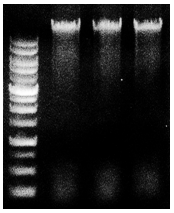


Figure 2. Genomic DNA (approximately 30 kb) was extracted using the Presto™ Mini gDNA Bacteria Kit. A *Bacillus subtilis* (1×10^9) culture (OD600=2, 1 ml) was harvested by centrifugation at 16,000 x g for 1 minute. A 5 µl aliquot of purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel. M = Geneaid 1 Kb DNA Ladder

Test	DNA Yield	260/280	260/230
1	12.28 µg	1.96	2.28
2	12.08 µg	1.97	2.30
3	12.56 µg	1.96	2.29

Related DNA Extraction Products

Genomic DNA Extraction and Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/101/300/301
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/300
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300

For additional product information please visit www.geneaid.com. Thank you!

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