Geneaid

Instruction Manual
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For Research Use Only

Presto™ 96 Well DNA Bacteria Advanced Kit

96GBBA02 (2 x 96 well plates/kit)
96GBBA04 (4 x 96 well plates/kit)
96GBBA10 (10 x 96 well plates/kit)

Advantages

Sample: up to 5 x 10^8 Gram-positive and Gram-negative bacteria cells
Yield: up to 10 µg of genomic DNA per well
Format: beadeating microtubes, Presto™ gDNA 96 Well Binding Plate
Operation Time: within 90 minutes
Elution Volume: 60~100 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™ 96 Well DNA Bacteria Advanced Kit is optimized for high-throughput genomic DNA purification from Gram (-) negative and Gram (+) positive bacterial cells. After directly adding bacteria samples into beadbeating microtubes pre-filled with zirconia/ceramic beads and lysis buffer, cells are efficiently lysed via bead beating instrument without enzymatic digestion. The cell lysate is then mixed with a binding buffer and the genomic DNA is bound by the membrane in the 96 well plate, followed by wash and elution. The entire procedure can be completed within 90 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA (approximately 20-30 kb) is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Presto™ 96 Well DNA Bacteria Advanced Kit is tested on a lot-to-lot basis by isolating DNA from *Escherichia coli* (5×10^8) culture. 5 µl from a 100 µl eluate of purified DNA is analyzed by electrophoresis on a 1% agarose gel.

Kit Components

Component	96GBBA02	96GBBA04	96GBBA10
GT Buffer	155 ml	155 ml x 2	155 ml x 4
PR Buffer	25 ml	50 ml	125 ml
GB Buffer[1] (Add Ethanol)	100 ml (100 ml)	100 ml x 2 (100 ml x 2)	240 ml x 2 (240 ml x 2)
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml (100 ml) 50 ml x 2 (200 ml x 2)
Elution Buffer	30 ml	75 ml	120 ml
RNase A (50 mg/ml)[3]	1 ml	1 ml x 2	1 ml x 5
Presto™ gDNA 96 Well Binding Plates	2	4	10
Beadbeating Microtubes (Racked)	2	2	2
Beadbeating Microtubes (8-strip)	N/A	12 x 2	12 x 8
Caps for Microtubes (8-strip)	24	48 x 1	72 x 1 48 x 1
96 Deep Well Plates[4]	2	2	2
Adhesive Film	6	12	30
0.35 ml Collection Plate	2	4	10

[1,2]Add absolute ethanol (see the bottle label for volume) to GB Buffer and Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Close the bottle tightly after each use to avoid ethanol evaporation.

[3]RNase A is shipped at room temperature and should be stored at 2-8ºC for extended periods.

[4]96 Deep Well Plates are reusable. After use, rinse with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O then autoclave.

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

Quick Protocol Diagram

Transfer 1.5 ml of cultured bacteria broth (up to 5 x 10^8 bacterial cells) into each well of the 96 Deep Well Plate. Prepare sample with RNase A and GT Buffer treatment.

Transfer samples to each well of beadbeating microtubes and lyse bacteria cells using a bead beating instrument.

DNA binding

Wash

Elution of pure DNA into 0.35 ml Collection Plate

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

Presto™ 96 Well DNA 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 GB Buffer and 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. 96 Deep Well Plates are reusable. After use, rinse with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O then autoclave.

Additional Requirements

Centrifuge with microplate buckets, 60ºC incubator, additional 96 Deep Well Plates, and absolute ethanol.

Centrifuge Protocol Procedure

1. Sample Collection

Transfer up to **1.5 ml of cultured bacteria broth or 10-20 mg (wet weight) of bacteria pellet (up to 5 x 10^8 bacterial cells)** into each well of a **96 Deep Well Plate**. Dry the top of the plate with paper towel then seal the plate with **Adhesive Film**. Centrifuge the 96 Deep Well Plate for 5 minutes at 3,000 x g to pellet the bacterial culture. Carefully remove the Adhesive Film from the 96 Deep Well Plate and remove the supernatant in each well by quickly inverting the plate.

2. Sample Preparation

Add **5 µl of RNase A (50 mg/ml) to 600 µl of GT Buffer per sample** and mix by inverting. **For 96 samples, add 500 µl of RNase A to 60 ml of GT Buffer** and mix by inverting. Transfer **600 µl of the mixture** into each well of the 96 Deep Well Plate containing the bacterial pellet. Resuspend the cell pellet by pipette until all traces of the cell pellet have been dissolved.

3. Lysis

After removing the lid and caps, transfer the re-suspended bacterial cells to the **Beadbeating Microtubes (Racked)**. Seal the microtubes with caps and lid. Secure the rack in a 96-well block/plate bead beating instrument (e.g., 2010 GenoGrinder or Qiagen TissueLyser II) and process the samples according to the manufacturer's intructions.

NOTE: Processing times may be as little as one minute when using a bead beating instrument so please follow the manufacturer's instructions for specific operating procedures.

4. Post Lysis

To eliminate foam caused by detergents, carefully remove the lid and caps then add **100 µl of PR Buffer** into each Beadbeating Microtube. Seal the tubes with new caps and close the rack lid. Vortex for 20-30 seconds then incubate at 4ºC for 5 minutes. Centrifuge at 3,000 x g for 5 minutes. Carefully remove the lid and caps then transfer **400 µl of the clarified supernatant** to each well of a new 96 Deep Well Plate.

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

5. Binding

Add **800 µl of GB Buffer (make sure ethanol was added)** into each well of the 96 Well Deep Plate. Dry the top of the plate with paper towel then seal with new Adhesive Film. Mix vigorously by shaking for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any solution from the Adhesive Film. Allow the centrifuge to reach 3,000 x g prior to stopping. Carefully remove the Adhesive Film. Place a **Presto™ 96 Well gDNA Binding Plate** on an empty **96 Deep Well Plate**. Transfer **600 µl of sample lysate** to each well of the Binding Plate, being careful not to get any lysate on the rims of the wells. Centrifuge together with the 96 Deep Well Plate at 3,000 x g for 5 minutes. Transfer the remaining sample lysate to each well of the Binding Plate, being careful not to get any lysate on the rims of the wells. Centrifuge together with the 96 Deep Well Plate at 3,000 x g for 5 minutes. Discard the flow-through then place the Binding Plate back on the 96 Deep Well Plate.

6. Wash

Add **400 µl of W1 Buffer** into each well of the Binding Plate then centrifuge together with the 96 Deep Well Plate at 3,000 x g for 5 minutes. Discard the flow-through then place the Binding Plate back on the 96 Deep Well Plate. Add **600 µl of Wash Buffer (make sure ethanol was added)** into each well of the Binding Plate then centrifuge together with the 96 Deep Well Plate at 3,000 x g for 5 minutes. Discard the flow-through then place the Binding Plate back on the 96 Deep Well Plate. Centrifuge together with the 96 Deep Well Plate at 3,000 x g for 10 minutes to dry the membrane.

7. Elution

Remove the Binding Plate from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the Binding Plate on **0.35 ml Collection Plate**. Add **100 µl of pre-heated Elution Buffer**[1], TE[2] or water[3] into the center of each well of the Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge together with the 0.35 ml Collection Plate at 3,000 x g for 5 minutes to elute the purified DNA. Seal the 0.35 ml Collection Plate with new Adhesive Film and store the purified DNA at -20ºC. NOTE: For maximum DNA yield, repeat the elution step by adding 100 µl of pre-heated Elution Buffer, TE or water to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuge again. If a higher DNA concentration is required, use 60 µl of pre-heated Elution Buffer then repeat the Elution step by adding the same 60 µl of Elution Buffer (which now contains the eluted DNA) to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuge again.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the CENTER of the well 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 well matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the CENTER of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Vacuum Protocol Procedure

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the **Presto™ gDNA 96 Well Binding Plate** in the binding top plate aperture. Attach the vacuum manifold to a vacuum source.

2. Sample Collection

Transfer up to **1.5 ml of cultured bacteria broth or 10-20 mg (wet weight) of bacteria pellet (up to 5 x 10^8 bacterial cells)** into each well of a **96 Deep Well Plate**. Dry the top of the plate with paper towel then seal the plate with **Adhesive Film**. Centrifuge the 96 Deep Well Plate for 5 minutes at 3,000 x g to pellet the bacterial culture. Carefully remove the Adhesive Film from the 96 Deep Well Plate and remove the supernatant in each well by quickly inverting the plate.

3. Sample Preparation

Add **5 µl of RNase A (50 mg/ml) to 600 µl of GT Buffer per sample** and mix by inverting. **For 96 samples, add 500 µl of RNase A to 60 ml of GT Buffer** and mix by inverting. Transfer 600 µl of the mixture into each well of the 96 Deep Well Plate containing the bacterial pellet. Resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved.

4. Lysis

After removing the lid and caps, transfer the re-suspended bacterial cells to the **Beadbeating Microtubes (Racked)**. Seal the microtubes with caps and lid. Secure the rack in a 96-well block/plate bead beating instrument (e.g., 2010 GenoGrinder or Qiagen TissueLyser II) and process the samples according to the manufacturer's instructions.

NOTE: Processing times may be as little as one minute when using a bead beating instrument so please follow the manufacturer's instructions for specific operating procedures.

5. Post Lysis

To eliminate foam caused by detergents, carefully remove the lid and caps then add **100 µl of PR Buffer** into each Beadbeating Microtube. Seal the tubes with new caps and close the rack lid. Vortex for 20-30 seconds then incubate at 4ºC for 5 minutes. Centrifuge the at 3,000 x g for 5 minutes. Carefully remove the lid and caps then transfer **400 µl of the clarified supernatant** to each well of a new 96 Deep Well Plate.

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

6. Binding

Add **800 µl of GB Buffer (make sure ethanol was added)** into each well of the 96 Well Deep Plate. Dry the top of the plate with paper towel then seal with new Adhesive Film. Mix vigorously by shaking for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any solution from the Adhesive Film. Allow the centrifuge to reach 3,000 x g prior to stopping. Carefully remove the Adhesive Film. Transfer **all of the sample lysate** into each well of the Binding Plate, being careful not to get any lysate on the rims of the wells.

NOTE: Seal unused wells of the Binding Plate with new adhesive film (not provided).

Apply vacuum at 15 inches Hg until samples pass through the Binding Plate then switch off the vacuum.

7. Wash

Add **400 µl of W1 Buffer** into each well of the Binding Plate. Apply vacuum at 15 inches Hg until **W1 Buffer** passes through the Binding Plate (approximately 10 seconds) then turn off the vacuum. Add **600 µl of Wash Buffer (make sure ethanol was added)** into each well of the Binding Plate. Apply vacuum at 15 inches Hg until Wash Buffer passes through the Binding Plate. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

8. Elution

Remove the Binding Plate from the manifold and blot the nozzles on clean, absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place collection plate spacer on the manifold base. Place a **0.35 ml Collection Plate** on top of the spacer. Place the binding top plate on the manifold base then place the Binding Plate in the binding top plate aperture. Add **100 µl of pre-heated Elution Buffer[1],** TE[2] or water[3] into the **CENTER** of each well of the Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes to elute the purified DNA then turn off the vacuum. Seal the 0.35 ml collection plate with new Adhesive Film and store the purified DNA at -20ºC.

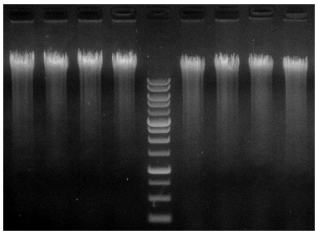
NOTE: For maximum DNA yield, repeat the elution step by adding 100 µl of pre-heated Elution Buffer, TE or water to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuge again. If a higher DNA concentration is required, use 60 µl of pre-heated Elution Buffer then repeat the Elution step by adding the same 60 µl of Elution Buffer (which now contains the eluted DNA) to each well of the Presto™ gDNA 96 Well Binding Plate then apply vacuum at 15 inches Hg for 5 minutes again. .

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the CENTER of the well 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 well matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the CENTER of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Presto™ 96 Well DNA Bacteria Advanced Kit Test Data



Sample	ng/ul	260/280	260/230	Yield
1	143.4	1.80	2.02	11.5
2	153.8	1.81	1.90	12.3
3	155.2	1.82	1.97	12.4
4	151.2	1.82	1.96	12.1
5	101.4	1.82	1.99	8.1
6	111.1	1.80	2.00	8.9
7	96.5	1.80	1.92	7.7
8	100.6	1.80	1.91	8.0

Figure 1. Genomic DNA was extracted from 20 mg of *Escherichia coli* and *Bacillus subtilis* pellet respectively using the Presto™ 96 Well DNA Bacteria Advanced Kit. 5 µl aliquots of purified genomic DNA from a 80 µl eluate was analyzed by electrophoresis on a 0.8% agarose gel.
M = Geneaid™ 1 Kb DNA Ladder
1-4: 5 x 10^8 *Escherichia coli* cells, 5-8: 5 x 10^8 *Bacillus subtilis* cells

Troubleshooting

Low Yield

Incorrect Sample Lysis

Homogenize bacteria samples in the Beadbeating Microtubes (Racked) using a 96-well block/plate bead beater (e.g., 2010 GenoGrinder or Qiagen TissueLyser II).

Incorrect DNA elution step

Use pre-heated Elution Buffer, TE or water (60ºC) to elute DNA. Ensure Elution Buffer, TE or water is added into the center of the matrix and is completely absorbed. If using water for elution, ensure the water pH is between 7.5 and 8.5. Elute twice to increase the DNA recovery.

Inappropriate buffer preparation

Add appropriate volume of absolute ethanol (see the bottle label) to the GB Buffer and Wash Buffer prior to use.

DNA contaminated with RNA

RNA carry-over

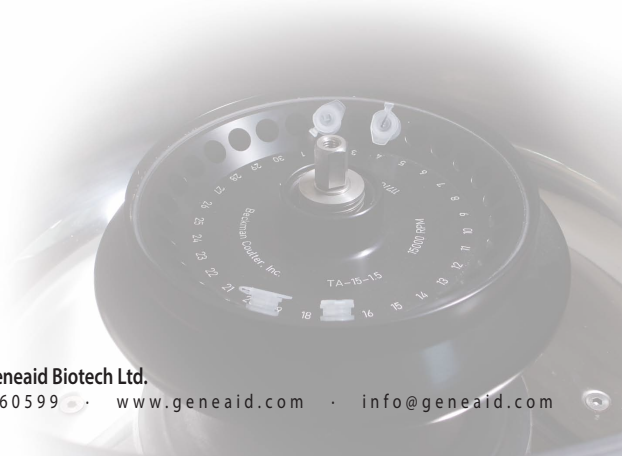
Add 5 µl of RNase A to 600 µl of GT Buffer per sample prior to resuspend the bacteria pellet.

Eluted DNA does not perform well in downstream applications

Residual ethanol contamination

Centrifuge protocol: following the wash step, dry the membrane the Binding Plate with additional centrifugation at 3,000 x g for 10 minutes to remove residual ethanol.

Vacuum protocol: following the wash step, dry the membrane of Binding Plate by applying vacuum at 15 inches Hg for an additional 10 minutes to remove residual ethanol.



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

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