Geneaid

Instruction Manual
Ver. 05.04.17
For Research Use Only

Presto™ 96 Well gDNA Bacteria Kit

96GBB02 (2 x 96 well plates/kit)
96GBB04 (4 x 96 well plates/kit)
96GBB10 (10 x 96 well plates/kit)

Advantages

Sample: up to 5 x 10^8 Gram-positive and Gram-negative bacteria cells
Yield: up to 5 µg of genomic DNA
Format: Presto™ gDNA 96 Well Binding Plate
Operation Time: within 90 minutes
Elution Volume: 100~200 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

Table of Contents

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

Introduction

The Presto™ 96 Well gDNA Bacteria Kit is optimized for high-throughput genomic and viral DNA purification from Gram (-) negative and Gram (+) positive bacterial cells. 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 binding plate. 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™ 96 Well gDNA Bacteria 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	96GBB02	96GBB04	96GBB10
Gram+ Buffer	50 ml	100 ml	220 ml
GT Buffer	60 ml	100 ml	200 ml
GB Buffer	60 ml	100 ml	155 ml x 1 60 ml x 1
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer[1] (Add Ethanol)	50 ml (200 ml)	50 ml x 2 (200 ml x 2)	50 ml x 4 (200 ml x 4)
Proteinase K[2] (Add ddH_2O)	11 mg x 4 (1.1 ml x 4)	65 mg x 1 (6.5 ml) 11 mg x 2 (1.1 ml x 2)	55 mg x 4 (5.5 ml x 4)
Lysozyme[3]	110 mg x 2	110 mg x 1 250 mg x 1	610 mg x 1 250 mg x 1
Elution Buffer	60 ml	100 ml	120 ml x 2
Presto™ gDNA 96 Well Binding Plates	2	4	10
Microtubes (Racked)	2	2	2
Microtubes (8-strip)	N/A	12 x 2	12 x 8
Caps for Microtubes (8-strip)	24	48	60 x 2
96 Deep Well Plates[4]	2	2	2
Adhesive Film	10	20	50

[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. Close the bottle tightly after each use to avoid ethanol evaporation.

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

[3]Lysozyme should be stored at -20ºC 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

Lyse bacteria cells with Proteinase K and Lysozyme

Incubate at 60ºC for 10 min. (Proteinase K) and/or 37ºC for 30 min. (Lysozyme)

DNA binding

Wash

Elution of pure DNA into Microtubes (Racked)

Presto™ 96 Well 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 to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

3. 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, 37ºC and 60ºC incubator, additional 96 Deep Well Plates, and absolute ethanol.

Sample Collection And Preparation

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.

2A. Gram (-) Negative Bacteria Preparation

Proteinase K Working Solution

A. Mix **20 µl of Proteinase K and 180 µl of GT Buffer** per sample by vortex.

B. For 96 samples, mix **2 ml of Proteinase K and 18 ml of GT Buffer** by vortex.

Add **200 µl of Proteinase K working solution** into each well of the 96 Deep Well Plate. Re-suspend the cell pellet by pipette until all traces of the pellet have been disolved. Dry the top of the plate with paper towel then seal with new Adhesive Film. Incubate at 60ºC for 10 minutes. For optimal lysis, mix occasionally or place the plate on a rocking platform during incubation.

NOTE: Pre-warm the required volume of **Elution Buffer (200 µl/ sample)** to 60ºC for DNA Elution.

Proceed to Optional RNA Removal Step and Lysis on Page 5 or the Vacuum Protocol Procedure on page 7.

2B. Gram (+) Positive Bacteria Preparation

Lysozyme Working Solution

A. Mix **0.8 mg of Lysozyme and 200 µl of Gram+ Buffer** per sample by vortex.

B. For 96 samples, mix **80 mg of Lysozyme and 20 ml of Gram+ Buffer** by vortex.

Add **200 µl of Lysozyme Working Solution** 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. Dry the top of the plate with paper towel. Seal the plate with new **Adhesive Film** then incubate at 37ºC for 30 minutes. For optimal lysis, mix occasionally or place the plate on a rocking platform during incubation. Briefly centrifuge the 96 Deep Well Plate 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 then add **20 µl of Proteinase K** into each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal with new **Adhesive Film**. Mix the samples by vortex then incubate at 60ºC for 10 minutes. For optimal lysis, mix occasionally or place the plate on a rocking platform during incubation.

Optional RNA Removal Step

Briefly centrifuge the 96 Deep Well Plate 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. Add 10 µl of RNase A (50 mg/ml) into each well of the 96 Deep Well Plate and mix well by pipette. Incubate the 96 Deep Well Plate for 10 minutes at room temperature.

NOTE: Pre-warm the required volume of **Elution Buffer (200 µl/ sample)** to 60ºC for DNA Elution. Proceed to page 7 if using the vacuum protocol procedure.

Centrifuge Protocol Procedure

1. Lysis

Briefly centrifuge the **96 Deep Well Plate** 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 then add **200 µl of GB Buffer** into each well. Dry the top of the plate with paper towel then seal with new **Adhesive Film**. Mix the sample by inverting the plate 10 times then incubate at 70ºC for 10 minutes. For optimal lysis, mix occasionally or place the plate on a rocking platform during incubation.

2. Binding

Briefly centrifuge the 96 Deep Well Plate 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 then add **200 µl of absolute ethanol** into each well. Dry the top of the plate with paper towel then seal with new **Adhesive Film**. Mix the sample by shaking the plate vigorously 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™ gDNA 96 Well Binding Plate** on a new **96 Deep Well Plate**. Transfer all of the 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.

3. 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 **500 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. Add another **500 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.

4. 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 **Microtubes (Racked)**. 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 Microtubes (Racked) at 3,000 x g for 5 minutes to elute the purified DNA. Seal the Microtubes (Racked) with new caps 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 50 µl of pre-heated Elution Buffer then repeat the Elution step by adding the same 50 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. Lysis

Briefly centrifuge the **96 Deep Well Plate** 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 then add **200 µl of GB Buffer** into each well. Dry the top of the plate with paper towel then seal with new **Adhesive Film**. Mix the sample by inverting the plate 10 times then incubate at 70ºC for 10 minutes. For optimal lysis, mix occasionally or place the plate on a rocking platform during incubation.

3. Binding

Briefly centrifuge the 96 Deep Well Plate 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 then add **200 µl of absolute ethanol** into each well. Dry the top of the plate with paper towel then seal with new Adhesive Film. Mix the sample by shaking the plate vigorously for 15-30 seconds. Briefly centrifuge the 96 Deep Well Plate 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 from the 96 Deep Well Plate and transfer all of the sample lysate into each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on the rims of the wells.

NOTE: Seal unused wells of the binding plate with adhesive film or tape.

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

4. 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 Binding Plate (approximately 10 seconds) then turn off the vacuum. Add **1 ml 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.

5. 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 **Microtubes (Racked)** on the manifold base. 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 Microtubes (Racked) with new caps and store the purified DNA at -20ºC.

NOTE: For maximum DNA yield, repeat the elution step by adding 200 µl of pre-heated Elution Buffer, TE or water 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 gDNA Bacteria Kit Test Data

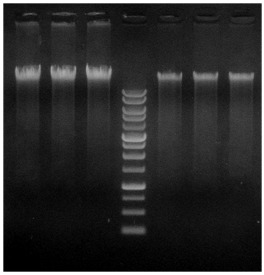


Figure 1. Genomic DNA was extracted from bacteria cells using the Presto™ 96 Well gDNA Bacteria Kit. The purified genomic DNA was eluted in 100 µl of Elution Buffer and 10 µl aliquots of the final sample (chosen from 6 random wells) were analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid™ 1 Kb DNA Ladder
1-3: 5 x 10^8 *Escherichia coli* cells, 4-6: 5 x 10^8 *Bacillus subtilis* cells

Sample	ng/µl	260/280	Yield (µg)
1	49.2	1.80	3.9
2	56.2	1.79	4.5
3	55.8	1.80	4.5
4	22.4	1.87	1.8
5	19.9	1.84	1.6
6	20.4	1.80	1.6

Troubleshooting

Low Yield

Incorrect Sample Lysis

When extracting genomic DNA from Gram (+) positive bacteria, prepare and use Lysozyme working solution to lyse bacteria cells. When extracting genomic DNA from Gram (-) negative bacteria, prepare and use Proteinase K working solution to lyse bacteria cells.

Too much starting material

Extract genomic DNA from up to 5 x 10^8 bacteria cells per reaction.

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.

DNA contaminated with RNA

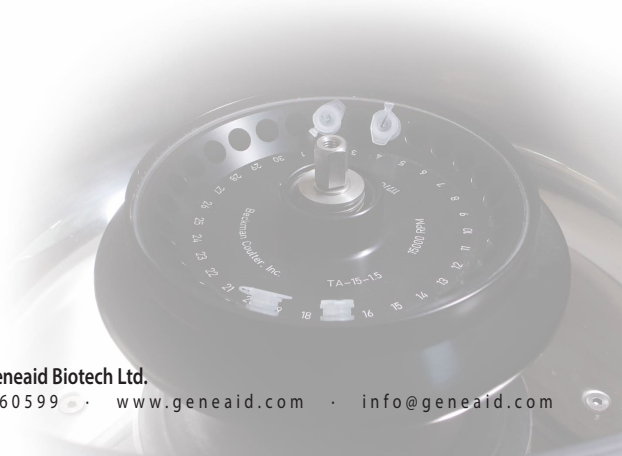
RNA carry-over

Perform RNA removal step.

Eluted DNA does not perform well in downstream applications

Residual ethanol contamination

Following the wash step, dry the Binding plate with additional centrifugation at 3,000 x g for 10 minutes to ensure the membrane is completely dry.



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

Geneaid

INTERNATIONAL

UKAS QUALITY MANAGEMENT

Registration Number 046

CERTIFICATE NO. QAIC/TW/50077

www.geneaid.com