Presto™ Mini gDNA Bacteria Kit Quick Protocol

For research use only

Catalogue Number

GBB004, GBB100/101, GBB300/301

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

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. 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.

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.

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.