Presto™ gDNA Bacteria Advanced Kit Quick Protocol

For research use only

Catalogue Number

GBBA004, GBBA100, GBBA300

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.

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.

Presto™ gDNA Bacteria Advanced Kit Protocol

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 (Beadbeating Tube Adapter (ADA006) can be purchased from Geneaid). 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[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE 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.

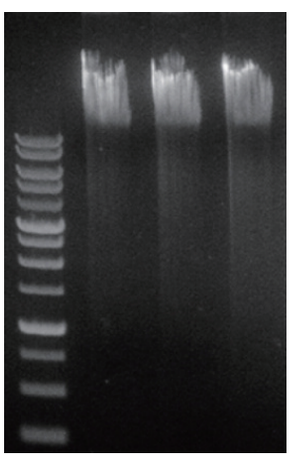
Components

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.

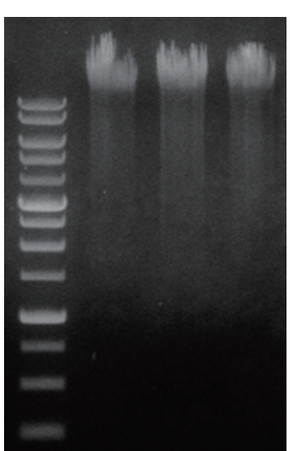
[2]RNase A is shipped at room temperature but should be stored at 4ºC for extended periods.

Presto™ gDNA Bacteria Advanced Kit Test Data



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 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	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

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