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Instruction Manual
Ver. 01.08.13
For Research Use Only

gBAC Mini DNA Bacteria Kit

IB47290 (4 Preparation Sample Kit)
IB47291 (100 Preparation Kit)
IB47292 (300 Preparation Kit)

Advantages

Sample: Gram (+) positive and Gram (-) negative bacterial cells
Yield: up to 30 µg of gDNA (1 x 10^9 *Escherichia coli*: 25-30 µg, 1 x 10^9 *Bacillus subtilis*: 10-15 µg)
Convenient: includes Gram+ Buffer for preparing lysozyme solutions and to speed up sample preparation
Format: genomic DNA spin columns
Operation Time: within 30 minutes
Elution Volume: 50-200 µl
Kit Storage: dry at room temperature (15-25°C) for up to 1 year

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Introduction

The gBAC Mini DNA Bacteria Kit is optimized for 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. Chaotropic salt is 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 gBAC Mini DNA Bacteria Kit is tested on a lot-to-lot basis by isolating DNA from *Escherichia coli* (1×10^9) culture (OD600=1.3, 1 ml) harvested by centrifugation at 16,000 x g for 1 minute. 10 µl from a 50 µl eluate of purified DNA is analyzed by electrophoresis on a 1% agarose gel.

Kit Components

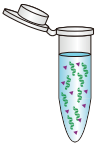
Component	IB47290	IB47291	IB47292
Gram+ Buffer[1]	1.5 ml	30 ml	75 ml
GT Buffer	1.5 ml	30 ml	75 ml
GB Buffer	2 ml	40 ml	100 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
GD Columns	4	100	300
2 ml Collection Tubes	8	200	600

[1]Add lysozyme to Gram+ Buffer immediately prior to use. Once lysozyme is mixed with Gram+ Buffer, the solution can be stored for 2 weeks at 4ºC.

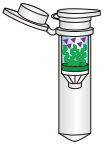
[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

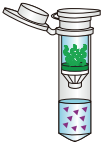
Quick Protocol Diagram



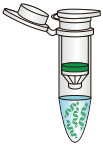
Sample preparation and cell lysis of bacterial cells



DNA binding to green membrane while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to green membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

gBAC Mini DNA Bacteria Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add lysozyme to Gram+ Buffer immediately prior to use. Residual Gram+ Buffer containing lysozyme should be stored at 4ºC for 2 weeks.

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.

Additional Requirements

absolute ethanol, microcentrifuge tubes, pipette tips, RNase A (10 mg/ml), 15 ml centrifuge tube (gram positive bacteria only), lysozyme (gram positive bacteria only)

Bacteria 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 **200 µl of GT Buffer** then re-suspend the cell pellet by shaking vigorously or pipette. Incubate at room temperature for 5 minutes then 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 (20 mg/ml) 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 shaking vigorously or pipette. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes. Proceed with Step 2 Lysis.

2. Lysis

Add **200 µl of GB Buffer** to the sample and mix by shaking vigorously for 5 seconds. Incubate at 60ºC 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 60ºC (for Step 5 DNA Elution).

Optional RNA Removal Step

Following 60ºC incubation, add 5 µl of RNase A (10 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.

7. 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. 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.

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.

Residual Ethanol Contamination.

Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual Ethanol Contamination.

Following the Wash Step, drying the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes will ensure the GD Column membrane is completely dry. Elute twice to increase yield.

gBAC Mini DNA Bacteria Kit Functional Test Data

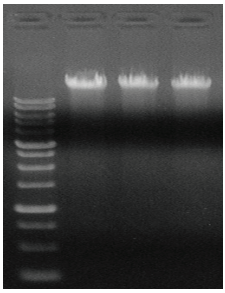


Figure 1. Genomic DNA (approximately 30 kb) was extracted using the gBAC Mini DNA Bacteria Kit. An *Escherichia coli* (1×10^9) culture (OD600=1.3, 1 ml) was harvested by centrifugation at 16,000 x g for 1 minute. The purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Test	DNA Yield	260/280	260/230
1	27.18 µg	2.03	2.57
2	28.84 µg	2.03	2.66
3	25.56 µg	2.04	2.62

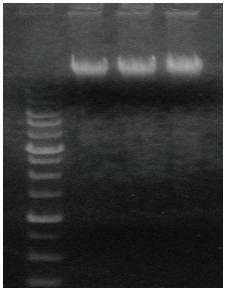


Figure 2. Genomic DNA (approximately 30 kb) was extracted using the gBAC Mini DNA Bacteria Kit. A *Bacillus subtilis* (1×10^9) culture (OD600=1.3, 1 ml) was harvested by centrifugation at 16,000 x g for 1 minute. The purified genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Test	DNA Yield	260/280	260/230
1	10.66 µg	1.90	2.10
2	11.88 µg	1.93	2.20
3	12.09 µg	1.92	2.14

Related DNA Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	4/10 x 96 preps	IB47151/152
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 preps	IB47040/050
Genomic DNA Extraction and Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47210
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMax Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100 preps	IB47230
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
96-Well Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
96-Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	IB47271/272

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

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