FastPure Bacteria DNA Isolation Mini Kit

Catalog #DC103

Version 8.1.0

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Introduction

FastPure Bacteria DNA Isolation Mini Kit is specially designed for genomic DNA isolation from bacteria of various origins (Gram-negative bacteria, Gram-positive bacteria). The kit uses silica gel membrane purification technology. No toxic reagents such as phenol chloroform are needed during the extraction process, and no time-consuming alcohol precipitation is required. It minimizes RNA, impurities, lipids and other inhibitory impurities to the utmost extent. The extracted genomic DNA has high purity and stable quality, and can be used in downstream experiments such as digestion, PCR, and Southern hybridization.

Package Information

Components	DC103–01 (100 rxn)
Buffer GA	40 ml
Proteinase K	44 mg
Proteinase K Dissolve Solution	4 ml
RNase A	400 µl
Buffer GB	40 ml
Buffer PB	26 ml
Buffer PW	40 ml
Elution Buffer	40 ml
FastPure gDNA Mini Columns III	100
Collection Tubes 2 ml	100

Note:

Buffer GA: provides a sample enzymatic environment;

Proteinase K: enzymatic hydrolysis of bacterial samples;

Proteinase K Dissolve Solution: Proteinase K Dilution Buffer;

RNase A: enzymatic hydrolysis of RNA in the sample;

Buffer GB: inactivated Proteinase K, balancing the column;

Buffer PB: removes impurities such as proteins and RNA remaining in DNA;

Buffer PW: removes residual salt ions in DNA;

Elution Buffer: elutes the binding DNA on the column;

Fastpure gDNA Mini Columns III: adsorbing genomic DNA;

Collection Tubes 2ml: filtrate collection tube.

Storage

Store RNase A and Proteinase K dry powder at -30℃ ~ -15℃. and transport at room temperature.
The other components should be stored at room temperature (15℃ - 25℃) and transported at room temperature.

Application

Genomic DNA isolation from < 1.0 x 10^9 Gram-negative bacteria or Gram-positive bacteria.

Additional Materials Required

Lysozyme (Vazyme, Cat.# DE103) (for Gram-positive bacteria), absolute ethanol, 1.5 ml centrifuge tube, water bath or metal bath.

Notes Before Use

◇ For the first time using, please refer to the label on the bottle, add the corresponding amount of absolute ethanol to Buffer PB and Buffer PW, mix before use.

◇ Check whether there is any precipitation in Buffer GA or Buffer GB before use. If there is precipitation, re-dissolve in 37℃ water bath and mix before use.

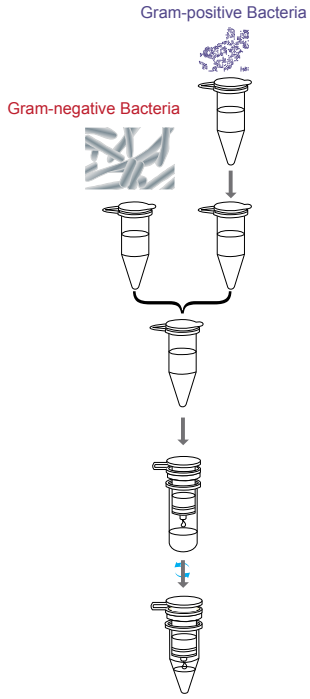
◇ Prepare the Protein Proteinase K into a final concentration of 20 mg/ml of PK working solution with Proteinase K Dissolve Solution before first time using.

◇ For samples from Gram-positive bacteria, please use Lysozyme to deal with the samples, with a final concentration of 20 mg/ml. Lysozyme is not available in this kit and can be purchased separately.

◇ The amount of sample processing should not exceed the compatibility of the kit, otherwise it will lead to a decrease in the amount of DNA extracted.

◇ All procedures are carried out at room temperature (15℃ - 25℃).

Overview of the Workflow



◇ Collect bacteria: Take 1-5 ml of bacterial culture (less than 1.0 x 10^9 bacteria), centrifuge at 10,000 rpm for 1 min, and discard the culture supernatant.

◇ Sample preparation: Add 180 µl of Lysozyme, water bath at 37℃ for 30 min (**Gram-positive bacteria**).
Add 230 µl of Buffer GA and mix by vortexing (**Gram-negative bacteria**).

Add 4 µl of RNase A and incubate at room temperature for 5 min (**Optional**).
Add 20 µl Proteinase K and mix by vortexing.

◇ Adjust column environment: Add 250 µl of Buffer GB, incubate in a water bath at 70℃ for 10 min.
Add 250 µl of absolute ethanol, mix by vortexing.

◇ Adsorption: Transfer the mixture to FastPure gDNA Mini Columns and centrifuge at 12,000 pm for 1 min.

◇ Removal of protein residue: Add 500 µl of Buffer PB, centrifuge at 12,000 rpm for 1 min.

◇ Removal of salt ion residue: Add 600 µl of Buffer PW and centrifuge at 12,000 rpm for 1 min (**Repeat once**).

◇ Removal of ethanol residue: empty column at 12,000 pm for 2 min.

◇ Genomic DNA elution: add 50 µl-100 µl of Elution Buffer, centrifuge at 12,000 rpm for 1 min.n

Protocol

1. Sample Preparation

◇ **For Gram-negative Bacteria**

1. Take 1 ml-5 ml of bacterial culture solution (less than 1.0 x 10^9 bacteria), centrifuge at 10,000 rpm (11,500 x g) for 1 min, and discard the culture supernatant.

▲ The number of bacteria can be measured by spectrophotometer, 1 OD_{600} is about 1.5 x 10^9 bacteria.

2. Add 230 µl of Buffer GA and vortex until the cells are completely suspended.

3. (Optional) If the RNA residue has a great influence on subsequent experiments, add 4 µl of RNase A to the mixture, vortex for 15 sec, and let stand for 5 min-15 min at room temperature.

4. Add 20 µl of Proteinase K and mix by vortexing.

5. Add 250 µl of Buffer GB, mix by vortexing, and incubate at 70℃ by water bath for 10 min.

▲ Buffer GB added may cause white precipitate, which will disappear when placed at 70℃, and will not affect subsequent experiments. If the solution does not become clear, that means the cell lysis is not complete, which may cause the DNA isolated in a small amount and impure.

6. Proceed to column purification.

◇ **For Gram-positive Bacteria**

1. Take 1 ml-5 ml of bacterial culture solution (less than 1.0 x 10^9 bacteria), centrifuge at 10,000 rpm (11,500 x g) for 1 min, discard the culture supernatant.

▲ The number of bacteria can be measured by a spectrophotometer. The 1 OD_{600} is about 1.5 x 10^9 bacteria.

2. Add 180 µl of lysozyme (self-prepared), shake to resuspend the bacteria, and bath at 37℃ for 30 min.

▲ Most bacterial have been completely lysed after 30 min water baths, but some bacteria with thick cell walls (such as Staphylococcus aureus) need to be treated for 1 h-2 h to completely lyse the wall. Please adjust the bath time according to different types of bacteria.

▲ Lysozyme dry powder should be prepared in buffer, otherwise it will cause it to be inactive, and its final working concentration is 20 mg/ml. The working buffer shoule be prepared as: 20 mM Tris, PH8.0; 2 mM Na-EDTA; 1.2% Triton X-100.

3. (Optional) If the RNA residue has a great influence on the subsequent experiments, add 4 µl of RNase A to the mixture, shake for 15 sec, vortex for 15 sec, and let stand for 5-15 min at room temperature.

4. Add 20 µl of Proteinase K, and mix by vortexing.

5. Add 250 µl of Buffer GB, mix by vortexing, and incubate at 70℃ by water bath for 10 min.

▲Buffer GB added may cause white precipitate, which will disappear when placed at 70℃, and will not affect subsequent experiments. If the solution does not become clear, that means the cell lysis is not complete, which may cause the DNA isolated in a small amount and impure.

6. Proceed to column purification.

2. Purification by Columns

1. Add 250 µl of absolute ethanol, mix by vortexing. If flocculent precipitate appears, centrifuge briefly to collect the liquid on the inner wall of the cap.

2. Transfer the above mixture to a FastPure gDNA Mini Column (the adsorption column was placed in a collection tube), centrifuged at 12,000 rpm (13,400 x g) for 1 min, and discard the filtrate.

3. Add 500 µl of Buffer PB (make sure that the absolute ethanol has been added before use) to the adsorption column, centrifuge at 12,000 rpm (13,400 x g) for 1 min, and discard the filtrate.

4. Add 600 µl of Buffer PW (make sure that the absolute ethanol has been added before use) to the adsorption column, centrifuge at 12,000 rpm (13,400 x g) for 1 min, and discard the filtrate.

5. Repeat the **Step 4**.

6. Place the column back into the collection tube and centrifuge at 12,000 rpm (13,400 x g) for 2 min.

▲ After the empty column is centrifuged, it can be opened for 2-5 min to completely evaporate the residual ethanol.

7. Transfer the adsorption column to a new 1.5 ml centrifuge tube (supplied), add 50-100 µl of Elution Buffer to the center of the adsorption column, place it at room temperature for 2-5 min, centrifuge at 12,000 rpm (13,400 x g) for 1 min.

Note: The following steps can help increase DNA yield:

▲ Preheat the Elution Buffer to 55℃ before elute;

▲ Repeat the elution step with the new Elution Buffer (this process can increase the yield but may reduce the concentration);

▲ If you want to increase the concentration of DNA, you can re-add the solution from the first elution to the adsorption column for elution.

8. Discard the adsorption column and store the DNA product at -20℃ to protect it forms degradation.

Troubleshooting

FAQ	Reasons	Solutions
Column Blocked	1. Excessive sample input.	Reduce the usage amount of sample, it should be less than 1.0 x 10^9 of bacteria.
	2. Indigestible substances in the digestion solution.	If there is obvious precipitate in the solution after the sample digestion, remove the undigested material by centrifuge at 12,000 rpm for 3 min.
Low DNA Yield	1. Insufficient sample input.	Adjust the amount of bacteria according to the bacterial culture condition. If the concentration is low, increase the amount of bacteria appropriately.
	2. Gram-positive bacteria are not completely lysed.	Increase the amount of Lysozyme appropriately or increase enzyme digestion time.
	3. The activity of Proteinase K is reduced or Proteinase K is inactivated.	Confirm proteinase K storage conditions or replace with new Proteinase K for digestion.
	4. Inappropriate Elution buffer.	Please use Elution Buffer. If eluted by ddH_2O or other eluent, confirm the pH is among pH 7.0 - pH8.5.
	5. Insufficient elution.	The eluent needs to be added to the center of the adsorption film; increase the elution volume or elution times.
	6. Absolute ethanol is not added to Buffer PB/PW.	Add the correct volume of absolute ethanol according to the label instructions.
Low DNA Purity	1. Insufficient sample lysis. The sample is not mixed with Buffer GB well.	Vortex to mix the sample with Buffer GB, or reduce the amount of sample.
	2. Protein/RNA contamination. The column was not washed by Buffer PB, or wash the column without correct centrifugal speed.	Try to ensure no precipitation in the supernatant used for column purification. Wash the column with Buffer PB according to the instructions.
	3. Ion pollution. Skip the Buffer PW washing step or wash the column only once.	Wash the column by Buffer PW twice according to the instructions, to remove residual ions as much as possible.
	4. Residual ethanol. Skip the step of centrifuging the empty tube after Buffer PW washing.	Centrifuge the empty tube after Buffer PW washing according to the instructions.