FastPure Bacteria DNA Isolation Mini Kit

DC103

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

This kit is mainly intended for genomic DNA extraction from bacteria (Gram-positive or Gram-negative) of a variety of sources. The kit is based on silica gel column purification technology that eliminates the need for extraction using phenol/chloroform organic solvents or time-consuming alcohol precipitation step. With this kit, RNA, proteins, lipids and other inhibitory impurities can be removed at the greatest extent. The DNA obtained can be directly used in PCR, qPCR, enzyme digestion and Southern blot, etc.

02/Components

Components	DC103-01 (100 rxns)
Buffer GA	40 ml
Proteinase K	2 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

Buffer GA: Provides an environment for proteolysis in samples.
Proteinase K: Catalyzes proteolysis in bacteria samples.
RNase A: Used to remove RNA.
Buffer GB: Inactivates Proteinase K and optimizes bingding conditions.
Buffer PB: Removes residual proteins, RNA, and other impurities in DNA.
Buffer PW: Removes residual salt ions in DNA.
Elution Buffer: Elutes DNA from the spin column.
FastPure gDNA Mini Columns III: Adsorb genomic DNA.
Collection Tubes 2 ml: Collect filtrate.

03/Storage

Store RNase A and Proteinase K at -30 ~ -15°C and transport at room temperature.
Store the other components at room temperature (15 ~ 25°C) and transport at room temperature.

04/Applications

<1.0 × 10^9 Gram-positive or Gram-negative bacteria.

05/Self-prepared Materials

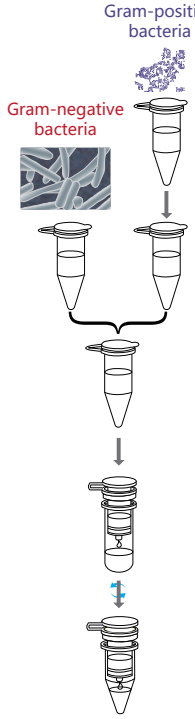
Lysozyme (Vazyme #DE103) (for Gram-positive bacteria), absolute ethanol, 1.5 ml centrifuge tubes, and water or metal baths.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Please add a specified amount of absolute ethanol to Buffer PB and Buffer PW according to the label before first use.
2. Check if there is any precipitation in Buffer GA and Buffer GB before use. If precipitates have formed, they can be re-dissolved in a 37°C water bath and mixed well before use.
3. For Gram-positive bacteria samples, self-prepared Lysozyme is required for the treatment of bacteria. The Lysozyme should be used at a final concentration of 20 mg/ml. Lysozyme is not provided with this kit but can be purchased separately.
4. The amounts of input shouldn't exceed range of application, which may result in decreased DNA yield.
5. Perform all steps at room temperature (15 ~ 25°C).

07/Mechanism & Workflow



◇ Bacteria collection: Take 1 - 5 ml of bacteria culture medium (less than 1.0 × 10^9 bacteria), centrifuge it at 10,000 rpm (11,500 × g) for 1 min, then discard the culture medium.

◇ Sample pretreatment: Add 180 µl of Lysozyme and incubate in a 37°C water bath for 30 min (**for Gram-positive bacteria**).
Add 230 µl of Buffer GA and mix by vortexing (**for Gram-negative bacteria**).
Add 20 µl of Proteinase K and mix by vortexing.

◇ Adjust binding conditions: Add 250 µl of Buffer GB and incubate in a 70°C water bath for 10 min.
Add 4 µl of RNase A and leave at room temperature for 5 - 15 min (optional).
Add 180 µl of absolute ethanol and mix by vortexing.

◇ Adsorption: Transfer the mixture to a FastPure gDNA Mini Columns III and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

◇ Removal of protein residue: Add 500 µl of Buffer PB and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

◇ Removal of salt ion residue: Add 600 µl of Buffer PW and centrifuge at 12,000 rpm (13,400 × g) for 1 min (repeat once).

◇ Removal of ethanol residue: Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.

◇ Elution of genomic DNA: Add 50 - 100 µl of Elution Buffer and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

08-1/Sample Pretreatment

◇ Gram-negative bacteria

1. Take 1 - 5 ml of bacteria culture medium (less than 1.0×10^9 bacteria), centrifuge it at 10,000 rpm (11,500 × g) for 1 min, then discard the culture medium.
   ▲ The quantity of bacteria can be measured with a spectrophotometer. 1 $OD_{600} \approx 1.5 \times 10^9$ bacteria.
2. Add 230 µl of Buffer GA and vortex to thoroughly suspend the bacteria.
3. Add 20 µl of Proteinase K and vortex for mixing.
4. Add 250 µl of Buffer GB, mix by vortexing, and incubate in a 70°C water bath for 10 min.
   ▲ The addition of Buffer GB may produce white precipitates. Generally, the precipitates will disappear during heating at 70°C and not affect subsequent experiments. If the solution does not become clear, it means that cells are not lysed thoroughly, and the extracted DNA may be insufficient and impure.
5. (Optional) If the RNA residue will greatly affect the subsequent experiments, add 4 µl of RNase A to the digested solution, vortex it for 15 sec, and leave it at room temperature for 5 - 15 min.
6. Proceed to 08-2/Column-Based Purification.

◇ Gram-positive bacteria

1. 1.Take 1 - 5 ml of bacteria culture medium (less than < 1.0×10^9 bacteria), centrifuge it at 10,000 rpm (11,500 × g) for 1 min, then discard the culture medium.
   ▲ The quantity of bacteria can be measured with a spectrophotometer. 1 $OD_{600} \approx 1.5 \times 10^9$ bacteria.
2. Add 180 µl of Lysozyme (self-prepared), shake for resuspending bacteria, and incubate in a 37°C water bath for 30 min.
   ▲ Most bacteria require 30 min water bathing for complete cell wall disruption, but some bacteria (e.g., *Staphylococcus aureus*) have thicker cell wall and need 1 - 2 h of water bathing. Please adjust the water bathing time according to the bacteria type.
   ▲ Lysozyme dry powder needs to be dissolved in a buffer at a final concentration of 20 mg/ml to show activity. The working buffer is prepared with 20 mM Tris (pH 8.0), 2 mM Na_2-EDTA, and 1.2% Triton X-100.
3. Add 20 µl of Proteinase K and mix by vortexing.
4. Add 250 µl of Buffer GB, mix by vortexing, and heat in a 70°C water bath for 10 min.
   ▲ The addition of Buffer GB may produce white precipitates. Generally, the precipitates will disappear during heating at 70°C and not affect subsequent experiments. If the solution does not become clear, it means that cells are not lysed thoroughly, and the extracted DNA may be insufficient and impure.
5. (Optional) If the RNA residue will greatly affect the subsequent experiments, add 4 µl of RNase A to the digested solution, vortex it for 15 sec, and leave it at room temperature for 5 - 15 min.
6. Proceed to 08-2/Column-Based Purification.

08-2/Column-Based Purification

1. Add 180 μl of absolute ethanol, vortex to mix well (floccules may appear), and collect the liquid on the tube cap by brief centrifugation.
2. Transfer the above mixture to a FastPure gDNA Mini Columns III (already fitted in a Collection Tube). Centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
3. Add 500 μl of Buffer PB (check whether absolute ethanol has been added before use) to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
4. Add 600 μl of Buffer PW (check whether absolute ethanol has been added before use) to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
5. Repeat Step 4.
6. Place the spin column back into the Collection Tube, and centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
   ▲ After centrifuging the empty column, air dry the column for 2 - 5 min for the residual ethanol to fully evaporate.
7. Transfer the spin column into a new 1.5 ml centrifuge tube (self-prepared), and add 50 - 100 μl of Elution Buffer to the center of the spin column membrane without touching the column. Incubate at room temperature for 2 - 5 min and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲ The following steps can help increase DNA yield:
   ▲ Pre-heat Elution Buffer to 55°C before elution.
   ▲ Repeat elution with newly taken Elution Buffer, which increases the DNA yield but reduces the concentration.
   ▲ To increase the DNA concentration, load the eluate from the first elution back onto the spin column for a second elution.
8. Discard the spin column and store the extracted DNA at -30 ~ -15°C to prevent degradation.

09/FAQ & Troubleshooting

FAQ	Cause	Solution
Clogged FastPure gDNA Columns III	1. Too much sample	Reduce the sample amount to less than 1.0×10^9 bacteria.
	2. Undigested substances in digested solution	If the solution resulting from sample digestion contains visible particulate matter, centrifuge the solution at 12,000 rpm (13,400 × g) for 3 min to remove the undigested substances.
No DNA extracted or low DNA yield	1. Too little sample	Determine the bacteria amount according to the bacterial culture situation. Some bacteria are lower in concentration after being cultured, so their usage amount can be increased appropriately.
	2. Incomplete cell wall disruption of Gram-positive bacteria	Increase the Lysozyme amount or extend the enzymatic digestion time as appropriate.
	3. Partial or complete activity loss of Proteinase K due to improper storage.	Check the storage conditions of Proteinase K or use new Proteinase K for digestion.
	4. Elution buffer issues	Please elute with Elution Buffer. If ddH_2O or another elution buffer is used, make sure that its pH is between 7.0 - 8.5.
	5. Incomplete elution	Add the elution buffer to the center of the adsorption membrane and increase the volume and number of elutions.
	6. Buffer PB/PW not supplemented with absolute ethanol	Add appropriate volumes of absolute ethanol as indicated on the label of the reagent bottle.
Low DNA Purity	1. Incomplete sample lysis	The sample and Buffer GB may not have been mixed thoroughly. Extract again, and this time mix the sample with Buffer GB thoroughly. Reduce the sample amount if it is too much.
	2. Protein and RNA contamination	Don't use Buffer PB to wash the spin column or use it with the correct centrifugation speed. Ensure there are basically no precipitates in the supernatant before loading it onto the spin column. The column must be washed with Buffer PB according to the Instructions for Use, and this step cannot be omitted. .
	3. Ion contamination	Don't wash the spin column with Buffer PW or just washed it once. Wash the column twice with Buffer PW as instructed to remove residual ions to the extent possible.
	4. Ethanol contamination	Have skipped empty column centrifugation after washing the column with Buffer PW. Centrifuge the empty column as Instructions for Use.

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