FastPure Cell/Tissue DNA Isolation Mini Kit

DC102

Vazyme

Instruction for Use

Version 24.2

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

This product is intended for the extraction of genomic DNA from 1 - 20 mg of animal tissues and less than 5 × 10^6 cultured cells. This 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. The whole extraction process takes only 30 - 60 min. The obtained DNA is ready for use in enzyme digestion, PCR, library preparation, virus DNA detection, etc.

02/Components

Components	DC102-01 (100 rxns)
Buffer GA	40 ml
Buffer GB	40 ml
RNase Solution	1.1 ml
Proteinase K	2 ml
Washing Buffer A	26 ml
Washing Buffer B	40 ml
Elution Buffer	40 ml
gDNA Columns	100
Collection Tubes 2 ml	100

Buffer GA: Provide an environment for sample lysis.

Buffer GB: Elute Proteinase K and provide an environment for binding.

RNase Solution: Degrade RNA in the sample.

Proteinase K: Lyse tissue sample.

Washing Buffer A: Remove proteins and other impurities from genomic DNA.

Washing Buffer B: Remove salt ions from genomic DNA.

Elution Buffer: Inactivate genomic DNA from the spin column.

gDNA Columns: Adsorb genomic DNA.

Collection Tubes 2 ml: Collect filtrate.

03/Storage

Store at 15 ~ 25°C and ship at room temperature.

04/Applications

1 - 20 mg of animal tissues.

<5 × 10^6 cultured cells.

Liquid samples.

05/Self-prepared Materials

1.5 ml sterile centrifuge tubes, absolute ethanol, water bath.

06/Notes

◇ Before the first use, add appropriate amounts of absolute ethanol to Washing Buffer A and Washing Buffer B according to the label, and mix well before use.

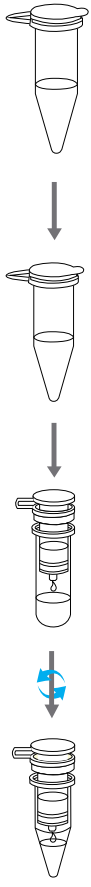
◇ Check for precipitates in Buffer GA, Buffer GB, and Washing Buffer A before use. Redissolve any precipitate in a 37°C water bath. Mix well before use.

◇ Avoid repeated freezing and thawing of samples, as this may lead to degradation of the extracted genomic DNA and reduce the extraction efficiency.

◇ Perform all steps at room temperature (15 ~ 25°C).

◇ The force acting on a centrifuge depends on the radius of a particular rotor. If an experiment is performed the same way every time, RCF (× g) must stay constant.

07/Mechanism & Workflow



Tissue:	**Cells:**	**Liquids:**
Cut 1 - 20 mg of animal tissue into pieces. 230 µl Buffer GA 20 µl Proteinase K Incubate in a 55°C water bath until complete lysis of tissue.	Collect up to 5 × 10^6 cells by centrifugation. 220 µl PBS 10 µl RNase Solution 20 µl Proteinase K Incubate at room temperature for at least 15 min.	250 µl sample 20 µl Proteinase K

Tissue samples: Add 250 µl of Buffer GB, vortex to mix well, and incubate in a 70°C water bath for 10 min. Cells and liquids: Add 250 µl of Buffer GB, vortex to mix well, and incubate in a 65°C water bath for 15 - 30 min.
Adjust binding conditions: Add 180 µl of absolute ethanol and mix by pipetting up and down.

Transfer the mixture to the spin column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
Removal of protein residue: Add 500 µl of Washing Buffer A to the spin column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
Removal of ion residue: Add 650 µl of Washing Buffer B to the spin column and centrifuge at 12,000 rpm (13,400 × g) for 1 min (twice).
Removal of ethanol residue: Centrifuge empty column at 12,000 rpm (13,400 × g) for 2 min.

Elution of genomic DNA: Use 30 - 100 µl of pre-heated Elution Buffer.

08/Experiment Process

08-1/Sample Processing

A. Digestion and Lysis of Animal Tissue (1 - 20 mg)

1. Transfer 1 - 20 mg of shredded or ground tissue (<10 mg of liver, spleen, or kidney tissue) to a 1.5 ml centrifuge tube. Add 230 µl of Buffer GA and 20 µl of Proteinase K, and mix well by vortexing.
   ▲ Excessive sample amounts lower DNA yield and purity. For DNA-rich tissues such as those from the liver, spleen, and kidney, use <10 mg of sample. For tissues low in DNA content, such as those from the muscle and skin, use 20 - 50 mg of sample, scale up the volumes of Buffer GA, Buffer GB, and absolute ethanol, and load the sample onto the spin column in multiple times as instructed in 08-2/Column Purification/Step 2.
2. Incubate the mixture in a 55°C water bath until complete lysis of the tissue.
   ▲ During sample digestion, mix by inverting the tube to facilitate lysis. Cut the tissue blocks as finely as possible to shorten the digestion time. Processing tissue samples by liquid nitrogen grinding or homogenization (mechanical or using a glass homogenizer) helps shorten the digestion time. The digestion time depends on the sample type and homogenization effect. Normal tissues require 0.5 - 3 h, while mouse tail samples require 6 - 8 h or overnight digestion.
3. (Optional) If residual RNA has a large impact on subsequent experiments, add 10 µl of RNase Solution to the lysate, mix well by inversion, and incubate at room temperature or 37°C for 15 - 60 min.
   ▲ The RNA digestion time depends on the sample type. For RNA-rich liver and kidney samples, the digestion time can be extended to 60 min.
4. If the lysate is turbid or contains visible particles, centrifuge at 12,000 rpm (13,400 × g) for 3 min and transfer the supernatant to a new 1.5 ml centrifuge tube.
5. Add 250 µl of Buffer GB to the lysate, mix by vortexing at the max speed for 20 sec, and incubate in a 70°C water bath for 10 min.
6. Proceed to 08-2/Column-based Purification.

B. Digestion and Lysis of Cultured Cells

1. Use no more than 5×10^6 cells. Centrifuge to pellet cells at 400 × g for 5 min and discard the supernatant. Add 220 µl of PBS, 10 µl of RNase Solution, and 20 µl of Proteinase K to resuspend the cells. Incubate the cells at room temperature for at least 15 min.
2. Add 250 µl of Buffer GB to the cell suspension, and mix well by vortexing. Incubate the cells in a 65°C water bath for 15 - 30 min.
3. Proceed to 08-2/Column-based Purification.

C. Liquid samples (anticoagulated blood, fresh blood, resuspension, etc.)

1. To 250 µl of saliva, milk, anticoagulated blood, or fresh blood, add 20 µl of Proteinase K, and mix well by vortexing.
2. Add 250 µl of Buffer GB to the sample, and mix well by vortexing. Incubate the sample in a 65°C water bath for 15 - 30 min.
3. Proceed to 08-2/Column-based Purification.

08-2/Column-based Purification

1. Add 180 µl of absolute ethanol to the lysate, and vortex for 15 - 20 sec to mix well.
   ▲ Precipitates may form when ethanol is added to liver or spleen tissue lysate. Mix well by pipetting up and down before adding the sample to the column.
2. Place gDNA Columns in a Collection Tube 2 ml. Transfer the mixture from the previous step (including precipitates) to the spin column. Centrifuge at 12,000 rpm (13,400 × g) for 1 min. In case of column clogging, centrifuge at the max speed for another 3 - 5 min. If the mixture exceeds 750 µl, add to the column in multiple times.
3. Discard the filtrate and place the gDNA Columns in the Collection Tube 2 ml. Add 500 µl of Washing Buffer A to the spin column. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
4. Discard the filtrate and place the gDNA Columns in the Collection Tube 2 ml. Add 650 µl of Washing Buffer B to the spin column. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
5. Repeat Step 4.
6. Discard the filtrate and place the gDNA Columns in the Collection Tube 2 ml. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
7. Place the gDNA Columns in a new 1.5 ml centrifuge tube. Add 30 - 100 µl of Elution Buffer (pre-heated to 70°C) to the center of the spin column membrane. Leave the column at room temperature for 3 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲ For DNA-rich tissue, add another 30 - 100 µl of Elution Buffer and elute again.
8. Discard the spin column and store the DNA at 2 ~ 8°C. For long-term storage, store at -30 ~ -15°C.

09/FAQ & Troubleshooting

◇ Column clogging

1. Too much sample: Reduce the sample amount. Use no more than 10 mg of sample rich in nucleic acids, such as liver and spleen samples.
2. Incomplete sample digestion: Grind or homogenize the sample in liquid nitrogen or with a homogenizer, or prolong Proteinase K digestion.
3. Incomplete sample lysis: The sample and Buffer GB are not mixed thoroughly. After adding Buffer GB, mix by inverting 3 - 5 times, then vortex at the maximum speed to mix the sample with Buffer GB thoroughly.

4. Insoluble substances in the lysate: If visible particles are still present after sample digestion, centrifuge at 12,000 rpm (13,400 × g) for 3 min to remove the undigested substances.

◇ Low DNA yield

1. Incomplete sample digestion: Prolong the digestion, or homogenize the sample with a glass homogenizer.
2. Low DNA content in the sample: Extract genomic DNA from liver/spleen tissue or other tissue rich in nucleic acids.
3. Washing Buffer is not supplemented with ethanol.
4. When precipitates form in the lysate after the addition of ethanol, pipette several times to break up the precipitates to increase the yield.
5. Incomplete elution: Add Elution Buffer to the center of the spin column membrane and increase the volume or number of times of elutions.

◇ Low DNA purity

1. Incomplete sample lysis: The sample and Buffer GB are not mixed thoroughly. Extract DNA again, mix well by inverting 3 - 5 times after adding Buffer GB, then vortex at the maximum speed to mix the sample with Buffer GB thoroughly.
2. Too much sample: Reduce the sample amount.
3. Complex samples: For tissue samples rich in metabolites, extract using a 1 × volume of phenol/chloroform after Buffer GA/Proteinase K digestion, then proceed to the next steps.
4. RNA-rich samples: For RNA-rich liver, kidney, and cultured cells, extend the digestion time with RNase Solution to 60 min.

Vazyme Biotech Co.,Ltd.
www.vazyme.com
400-600-9335 (China) +86 400-168-5000 (Global)
support@vazyme.com