FastPure® Cell/Tissue DNA Isolation Mini Kit

DC102

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Instruction for Use

Version 21.1

Contents

01/Product Description

This product is suitable for extracting genomic DNA from 1 - 20 mg of animal tissues and cultured cells (<5 × 10^6). 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 during the extraction process. The entire extraction process only lasts for 30 - 60 min. The obtained DNA can be directly used in restriction enzyme digestion, PCR, library preparation, viral 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 enzymolysis environment;

RNase Solution: RNA digestion;

Washing Buffer A: Remove impurities such as protein;

Elution Buffer: Elute the bound DNA;

Buffer GB: Inactivate Proteinase K and provide the column environment;

Proteinase K: Tissue sample lysis;

Washing Buffer B: Remove salt;

gDNA Columns: Genomic DNA adsorption column;

Collection Tubes 2 ml: Filtrate collection tubes.

03/Storage

Store the Proteinase K and RNase Solution at -30 ~ -15℃ and transport at room temperature; Store the other components at 15 ~ 25℃, and transport at room temperature.

04/Applications

1 - 20 mg of animal tissue

Cultured cells (<5 × 10^6)

Liquid samples

05/Self-prepared Materials

1.5 ml sterilized centrifuge tube; absolute ethanol; water bath.

06/Notes

1. Add the corresponding amount of absolute ethanol indicated in the bottle label of Washing Buffer A and Washing Buffer B , and mix thoroughly before use.
2. Before starting the procedure, check whether precipitates have formed in Buffer GA, Buffer GB and Washing Buffer A. If necessary, dissolve by heating at 37℃ in a water bath with gentle agitation.
3. Avoid repeated freezing and thawing of the samples, as this can result in degradation of the extracted genomic DNA and a reduction in extraction efficiency.
4. All operating procedures must be carried out at room temperature (15 ~ 25℃).

07/Mechanism & Workflow

Tissue samples:	**Cell samples:**	**Liquid samples:**
1 - 20 mg of chopped animal tissues 230 µl Buffer GA 20 µl Proteinase K Incubate at 55℃ in a water bath until the tissues are completely lysed	Collect the cells by centrifugation 220 µl PBS 10 µl RNase Solution 20 µl Proteinase K Incubate at room temperature for over 15 min.	250 µl sample 20 µl Proteinase K

Tissue samples: Add 250 µl Buffer GB, mix by vortexing, and incubate at 70℃ in a water bath for 10 min.
Cell and liquid samples: Add 250 µl Buffer GB, mix by vortexing, and incubate at 65℃ in a water bath for 15 - 30 min.

Adjust the column environment: Add 180 µl absolute ethanol and mix by pipetting.

Transfer the mixtures to the adsorption column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
Remove the protein residue: Add 500 µl Washing Buffer A to the adsorption column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
Remove the ionic residues: Add 650 µl Washing Buffer B to the adsorption column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
(Twice)
Remove the ethanol residue: Centrifuge the empty tube at 12,000 rpm (13,400 × g) for 2 min.

Genome elution: Add 30 - 100 µl pre-heated Elution Buffer.

08/Experiment Process

08-1/Sample Processing

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

1. Place 1 - 20 mg of chopped or grind tissues (or less than 10 mg of liver, spleen and kidney) in a 1.5 ml centrifuge tube. Add 230 µl Buffer GA and 20 µl Proteinase K and mix by vortexing.
   ▲Excessive amount of samples will result in a reduction in DNA yield and purity. Samples such as liver, spleen and kidney are rich in DNA, and the samples shall be less than 10 mg in weight. For tissues with low DNA contents such as muscle and skin, samples can be increased to 20 - 50 mg in weight while proportionally increasing the amount of Buffer GA, Buffer GB, and absolute ethanol.
2. Incubate at 55℃ in a water bath until the tissues are completely lysed.
   ▲Mix by inversion to promote the lysis process. Chop the tissues into pieces to shorten the digestion time. Grind with liquid nitrogen, mechanical homogenization, glass homogenization and other methods for tissue samples processing can shorten the digestion time. Digestion time is dependent on the sample type and homogenization results. In general, 0.5 - 3 h is required for tissue samples, 6 - 8 h or overnight digestion is required for mouse tails.
3. (Optional) If the RNA residues significantly affect subsequent experiments, add 10 µl RNase Solution to the digestion solution, mix by inversion, and incubate at room temperature or 37℃ for 15 - 60 min.
   ▲The RNA digestion time is dependent on the sample type. Liver and kidney are rich in RNA, so please increase digestion time to 60 min.
4. If the digestion solution is turbid or contains 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 Buffer GB to the digestion solution, and mix by vortexing at the highest speed for 20 sec, then incubate at 70℃ in a water bath for 10 min.
6. Proceed to 08-2 Column Purification.

B. Digestion and Lysis of Cultured Cells

1. The total number of cells must not exceed 5 × 10^6. Collect the cells by centrifugation at 400 × g for 5 min, and discard the supernatant. Add 220 µl PBS, 10 µl RNase Solution, and 20 µl Proteinase K to the sample, then resuspend the cells. Incubate at room temperature for over 15 min.
2. Add 250 µl of Buffer GB to the cell suspension and mix by vortexing. Incubate at 65℃ in a water bath for 15 - 30 min.
3. Proceed to 08-2 Column Purification.

C. Liquid Samples (Anticoagulated Blood, Blood Water, Resuspensions, etc.)

1. Pipet 250 µl of sample (such as saliva, milk, anticoagulated blood and blood water) into a 1.5 ml sterilized centrifuge tube. Add 20 µl of Proteinase K and mix by vortexing.
2. Add 250 µl Buffer GB to the sample and mix by vortexing. Incubate at 65℃ in a water bath for 15 - 30 min.
3. Proceed to 08-2 Column Purification.

08-2/Column Purification

1. Add 180 µl absolute ethanol to the digestion solution, and mix by vortexing for 15 - 20 sec.
   ▲ If precipitates form in liver or spleen digestion solution after adding absolute ethanol, please pipette to mix the precipitates before passing through the column.
2. Place the gDNA Columns in 2 ml collection tubes. Transfer the mixture (including precipitates) from the last step to the adsorption column. Centrifuge at 12,000 rpm (13,400 × g) for 1 min. If the column becomes clogged, centrifuge again at the highest speed for 3 - 5 min. If the volume of the mixture is higher than 750 µl, it must be repeatedly passed through the column.
3. Discard the flow-through and place the adsorption column in a collection tube. Add 500 µl Washing Buffer A to the adsorption column. Centrifuge at 12,000 rpm (13,400× g) for 1 min.
4. Discard the flow-through and place the adsorption column in the collection tube. Add 650 µl washing buffer B to the adsorption column. Centrifuge at 12,000 rpm (13,400× g) for 1 min.
5. Repeat Step 4.
6. Discard the flow-through and place the adsorption column in a collection tube. Centrifuge the empty tube at 12,000 rpm (13,400 × g) for 2 min.
7. Place the adsorption column in a new 1.5 ml centrifuge tube. Add 30 - 100 µl Elution Buffer pre-heated to 70℃ to the center of the adsorption column membrane, and incubate at room temperature for 3 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲For DNA-rich tissues, add another 30 - 100 µl Elution Buffer and repeat the elution.
8. Discard the adsorption column and store the DNA at 2 ~ 8℃, or store at -30 ~ -15℃ for longterm storage.

09/FAQ & Troubleshooting

◇ Clogged Column

Excessive amount of sample: Reduce the sample amount. It should not exceed 10 mg in weight for nucleic acid-rich samples such as the liver and spleen.

Insufficient sample digestion: Use liquid nitrogen or a homogenizer to grind and homogenize the sample, or increase the Proteinase K digestion time.

Insufficient lysis of the sample: The sample and Buffer GB were not sufficiently mixed. After adding Buffer GB, mix by inverting 3 to 5 times, and mix by vortexing at the highest speed to thoroughly mix the sample and Buffer GB.

Undissolved substances in the digestion solution: If there are still distinct particles after the sample was digested, centrifuge at 12,000 rpm (13,400 × g) for 3 min to remove the undigested substances.

◇ Low DNA Yield

Insufficient sample digestion: Increase digestion time to allow for complete digestion of the sample, or homogenize the sample using a glass homogenizer.

Low DNA contents: Use nucleic acid-rich tissues such as liver and spleen for genomic DNA extraction.

Ethanol was not added to the Washing Buffer.

When ethanol is added to the digestion solution and precipitation occurs, the yield can be increased by pipetting the solution several times to break up the precipitates.

Insufficient elution: The eluent must be added to the center of the adsorption column or increase the elution volume or times.

◇ Low DNA Purity

Insufficient lysis of the sample: The sample and Buffer GB were not sufficiently mixed. Re-extract, add Buffer GB and mix by inverting 3 to 5 times, then mix by vortexing at the highest speed to thoroughly mix the sample and buffer GB.

Excessive amount of sample: Reduce the amount of sample.

Complex samples: For certain tissues rich in metabolites, after digestion of the sample with buffer GA/Proteinase K, extract with an equal volume of phenol/chloroform before proceeding.

RNA-rich samples: For RNA-rich samples (liver, kidney and RNA-rich cultured cells), the digestion time of the RNase Solution can be increased to 60 min.