Genomic DNA Isolation Kit (Fresh tissue)

Cat No. PDC11-0100
Size: 100 Reactions
Sample: 30 mg of fresh animal tissue
Format: spin column
Column capacity: up to 50 µg
Operation time: within 60 minutes

Description

The Genomic DNA Isolation Kit (Fresh tissue) is designed specifically for genomic DNA isolation from animal tissue samples. This unique buffer system ensures total DNA with high yield and good quality from samples. The spin column system is designed to purify and concentrate DNA products which have been previously isolated using buffers. The entire procedure can be completed in 1 hour without phenol / chloroform extraction. Purified DNA is suitable for use in PCR or other enzymatic reactions.

Feature

- Delivering high-quality genomic DNA with the fast procedure
- Ready-to-use gnomic DNA for high performance in any downstream application
- Highly purified and high yield genomic DNA can be extracted from various samples
- Optimized lysis buffer for the efficient lysis
- Designed to rapidly purify high-quality DNA using spin column format

Application

- Gene cloning
- Southern blotting
- PCR
- SNP genotyping

Kit Contents

Contents	
Buffer TL	25 ml
Buffer TP	12 ml
Buffer W1	45 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)
Buffer BE	10 ml
Column TC	100 pcs
Collection Tubes	100 pcs

Quality Control

The quality of the Genomic DNA Isolation Kit (Fresh tissue) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Mortar and pestle
- Proteinase K (10 mg/ml)
- Absolute ethanol
- Micropestle
- RNase A (50 mg/ml)
- Isopropanol
- Microcentrifuge tubes

Protocol

Fresh Tissue

Step 1 Sample Preparation

1. Cut off 30 mg of the fresh animal tissue and grind the sample under liquid nitrogen to a fine powder using a mortar and pestle and transfer it to a 1.5 ml microcentrifuge tube or transfer the tissue to a 1.5 ml microcentrifuge tube and use the micropestle to grind the tissue to a pulp.

Step 2 Lysis

1. Add 300 µl of the Buffer TL and 20 µl of the Proteinase K(10mg/ml) to the tube from Step 1.
2. Incubate at 60°C for 30 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 5 minutes. #Pre-heat the Elution Buffer to 60°C for Step 6 DNA Elution.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (50 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal

1. Add 100 µl of the Buffer TP to the sample from Step 2 and shake vigorously.
2. Centrifuge at 12,000 x g for 1 minute.(Don`t over 1 minute)

Step 4 DNA Binding

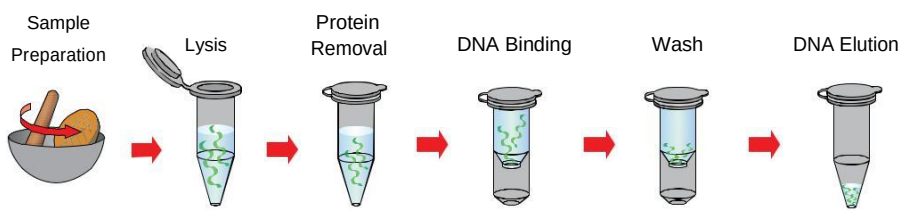
1. Place a Column TC in a 2 ml Collection Tube.
2. Transfer the clear supernatant completely from the previous step to the Column TC.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the Column TC back in the same Collection Tube.

Step 5 Wash

1. Add 400 µl of the Buffer W1 into the Column TC.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the Column TC back into the same Collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the Column TC.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the Column TC back into the same Collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 6 DNA Elution

1. Transfer the dried Column TC to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of the Pre-Heated Buffer BE or TE into the center of the column matrix.
3. Let stand at 60°C for 3 minutes.
4. Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the genomic DNA with the kit.

Problem	Cause	Solution
Low yield of DNA	Incomplete lysed sample	Use the appropriate method for the lysate preparation based on the amount of the starting materials.
		Increase the digestion time.
		Make sure that the tissue is completely immersed in the Buffer TL.
	Ethanol not added to Buffer W2	Add 60 ml of the ethanol (96–100%) to Buffer W2, and shake before use
	Incorrect elution conditions	Perform incubation at 75°C for 3 minutes with Buffer BE before centrifugation. To recover more DNA, perform a second elution step.
	Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample at -80°C or in liquid nitrogen. The yield and quality of DNA isolated depends on the type and age of the starting material.
DNA degrade	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Use a new sample for the DNA isolation. Perform the extraction of the fresh material when possible.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
		Maintain a sterile work environment to avoid contamination from DNases.

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Check buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- Add 60 ml of the ethanol (96–100%) to Buffer W2, and shake before use.
- Buffers W1 contain irritants. Wear gloves when handling these buffers.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.