Genomic DNA Isolation Kit (Fresh tissue)

Cat. No.: SN026-0100 Size: 100 Reactions
Cat. No.: SN026-0004 Size: 4 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 samples which have been previously isolated using buffers. The entire procedure can be completed in one hour without phenol / chloroform extraction. Purified DNA is suitable for PCR or other enzymatic reactions.

Features

- Ready-to-use genomic DNA for high performance in any downstream application.
- Optimized lysis buffer for the efficient lysis.
- Rapidly purify high-quality DNA using spin column format.

Applications

- Gene cloning.
- Southern blotting.
- PCR.
- SNP genotyping.

Kit Contents

Contents	SN026-0100	SN026-0004
Buffer TL	35 ml X 1 vial	1.5 ml X 1 vial
Buffer TP	15 ml X 1 vial	0.5 ml X 1 vial
Buffer W1	45 ml X 1 vial	2 ml X 1 vial
Buffer W2 (Add ethanol)	15 ml X 1 vial (60 ml X 1 vial)	300 µl X2 vials (1.2 ml X 2 vials)
Buffer BE	10 ml X 1 vial	1 ml X 1 vial
TC Column	50 pieces X 2 bags	4 pieces X 1 bag
Colletion Tube	50 pieces X 2 bags	

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

- Proteinase K (10 mg/ml)
- Water bath/ Dry bath
- Absolute ethanol
- Mortar and pestle or micropestle
- RNase A (50 mg/ml) (Optional)
- Isopropanol
- Ethanol (96-100%)
- 1.5 ml microcentrifuge tubes

Buffer Preparation

- TE buffer, pH8.0 (Selective): 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA

Genomic DNA Isolation Kit (Fresh tissue) Protocol

Step 1 Sample Preparation

1. Cut off the fresh animal tissue (up to 30 mg) 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 Buffer BE to 60°C for Step 6 DNA Elution.
4. Optional Step:
   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 Buffer TP to the sample from Step 2 and shake vigorously.
2. Centrifuge at 14,000 x g for 3 minute. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
3. Add 300 µl of absolute ethanol to the sample lysate and shake vigorously (break up any precipitate by pipetting and does not let any pellet remain inside the pipette tip after you are done).

Step 4 DNA Binding

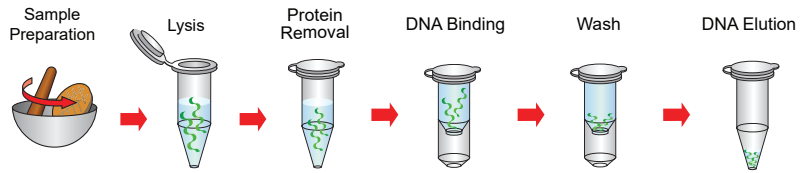
1. Place a TC Column in a 2 ml Collection Tube.
2. Transfer the clear supernatant completely from the previous step to the TC Column.
3. Centrifuge at 14,000 x g for 30 seconds.
4. Discard the flow-through and place the TC Column back in the 2 ml Collection Tube.

Step 5 Wash

1. Add 400 µl of the Buffer W1 into the TC Column.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the TC Column back into the same Collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the TC Column.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the TC Column 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 TC Column to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of the Pre-Heated Buffer BE or TE buffer (not provided) 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 you did genomic DNA isolation 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 ethanol (96-100%) to Buffer W2, and shake before use.
	Incorrect elution conditions	Perform incubation at 60°C for 3 minutes with Buffer BE or TE buffer 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 is 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.

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM201-0100	Hot Star SUPERMIX	100 Reactions
SM255-0100	Ultrapure Proteinase K	100 mg
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml
SD010-R500	1 Kb DNA Ladder RTU	500 µl
SD013-R500	XLarge DNA Ladder RTU	500 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml

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.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- All products are for research use only.