Genomic DNA Isolation Dual Kit (Tissue)

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For research use only

Sample: 30~100 mg of fresh animal tissue or 25 mg of paraffin-embedded tissue

Format: Reagent and spin column

Operation time: within 60 minutes

Introduction

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

Kit Contents

Catalog No.	DT0004	DT0100	DT0200	DT0300
DG Buffer	4 ml	100 ml	100 ml×2	100 ml×3
BD Buffer	4 ml	100 ml	100 ml×2	100 ml×3
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300μl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml×2 (100 ml)×2
EL Buffer	1 ml	10 ml	20 ml	30 ml
DG Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the Genomic DNA Isolation Dual Kit (Tissue) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

RNase A (50 mg/ml), Isopropanol, absolute ethanol, mortar and pestle, chloroform, microcentrifuge tubes.

NOTE

★ Add ethanol (96–100%) to Buffer W2, **shake before use** (see bottle label for volume).

★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

★ Buffers W1 contain irritants. Wear gloves when handling these buffers.

Protocol

Step 1 Sample Preparation (Fresh tissue)

- Cut off 100 mg of fresh animal tissue and grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 1 Sample Preparation (Paraffin-embedded tissue)

- Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
- Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.
- Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
- Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
- Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
- Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
- Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
- Open the tube and Incubate at 37ºC for 15 minutes to evaporate any ethanol residue. Proceed with the Lysis Step.

Step 2 Lysis

- Add **1 ml of DG reagent and 0.5 µl of RNase A (50 mg/ml)** to the sample from Step 1 and grind the sample until it is completely dissolved.
- Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. Incubate at 75ºC for 30 minutes.(invert the tube every 10 minutes)
- Centrifuge at 14-16,000 x g for 5 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Phase Separation

- Add 600 µl of chloroform to the supernatant from Step 2. Shake vigorously and then centrifuge at 14-16,000 x g for 10 minutes.
- Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
- **Repeat the Phase Separation Step until the interphase becomes clear then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.**

  NOTE: The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.

Step 4 DNA Precipitation

- Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 3.
- Mix the sample by inverting gently and let stand for 5 minutes at room temperature (DNA precipitation can be increased with extended standing time).
- Centrifuge at 14-16,000 x g for 15 minutes. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
- Centrifuge at 14-16,000 x g for 5 minutes. **Completely discard the supernatant** and re-suspend the pellets in 200 µl of TE buffer or ddH_2O.
- Incubate for 10 minutes at 75ºC to dissolve the pellet.
- If more pure DNA is required, perform this optional DNA Pure Protocol.

DNA Purification Protocol

#Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

#Pre-heat the Elution Buffer to 75ºC prior to use

Step 1 Sample Preparation

- Add **1 ml of BD Buffer** to the sample which have been previously isolated using reagents and shake vigorously.
- Place a **DG Column** in a **Collection Tube**.

Step 2 DNA Binding

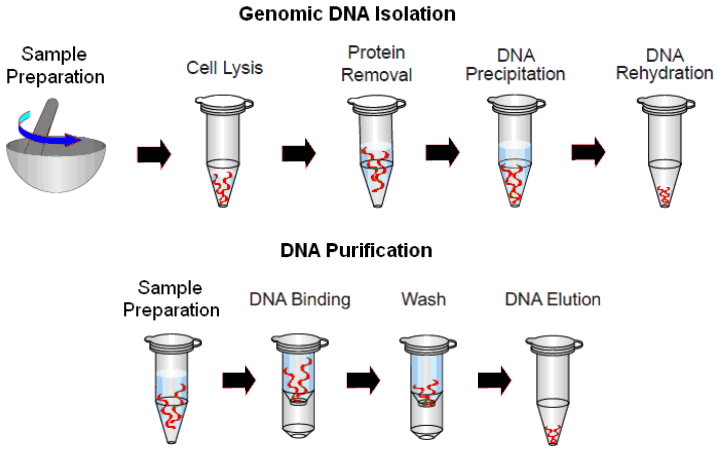
- Transfer the sample mixture from the previous step to the **DG Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and transfer the remaining mixture to the same **DG Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the **DG Column** back in the **Collection Tube**.

Step 3 Wash

- Add **400 µl of W1 Buffer** to the **DG Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the **DG Column** back in the **2 ml Collection Tube**.
- Add **600 µl of W2 Buffer** (ethanol added) into the **DG Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the **DG Column** back in the **2 ml Collection Tube**.
- Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

Step 4 DNA Elution

- Transfer the dried **DG Column** to a new 1.5 ml microcentrifuge tube.
- Add **50-200 µl of Pre-Heated EL Buffer** or TE into the center of the column matrix.
- Let stand at 75ºC for 5 minutes.
- Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	Use only the required range or amount of starting materials to prepare the lysates.
		Increase the digestion time.
		Make sure that the tissue is completely immersed in the DG Buffer.
	Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the W2 Buffer prior to use.
	Incorrect elution conditions	Perform incubation at 75°C for 3 minutes with Buffer EL before centrifugation. To recover more DNA, repeat the elution step.
	Poor starting material quality	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 degradation	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample. Use a new sample for DNA isolation. Perform the extraction using fresh materials whenever possible.
	DNase contaminantion	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents). Use fresh TAE or TBE electrophoresis buffer.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol	If residual solution is seen in the purification column after washing the column with Buffer W2, empty the collection tube and re-spin the column for an additional 1 min. at the maximum speed (≥12000 x g).
	Purified DNA contains residual salt	Use the Wash Buffers in their correct order. Always wash the purification column with Buffer W1 first and then proceed to the wash step with Buffer W2.