Genomic DNA Extraction Kit (Tissue)

For research use only

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

Format: spin column **Operation time:** within 60 minutes

Introduction

The BioDiamond Genomic DNA Extraction Kit (Tissue) was designed specifically for genomic DNA isolation from animal tissue samples. Its unique buffer system ensures total DNA with high yield and good quality from samples while its spin column system purifies or concentrates DNA products previously isolated with buffers. The entire procedure can be completed within 1 hour without phenol / chloroform extraction. Purified DNA is then suitable for use in PCR or other enzymatic reactions.

Kit Contents

Catalog No.	DMGTS100	DMGTS300
TL Buffer	35 ml	95 ml
TP Buffer	12 ml	35 ml
W1 Buffer	45 ml	125 ml
W2 Buffer (Add Ethanol)	15 ml (60 ml)	50 ml (200 ml)
EL Buffer	10 ml	30 ml
TC Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs

Quality Control

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

Additional requirements

∗RNase A (50 mg/ml) ∗Proteinase K (10 mg/ml) ∗mortar and pestle ∗absolute ethanol ∗microcentrifuge tubes ∗micropestle.

NOTE

★ Add ethanol (96–100%) to Buffer W2, **shaking 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 30 mg of 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.

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 **300 µl of TL Buffer** and **20 µl of Proteinase K(10mg/ml)** to the tube from Step 1.

◆ Incubate at 60˚C for 30 minutes and invert the tube every 5 minutes. If the lysate has not become totally clear at the 30 minute mark, use a micropestle to grind the remaining pellet and place the sample back at 60˚C until it is clear. #Pre-heat the Elution Buffer to 60ºC for Step 6 DNA Elution.

Optional Step:

RNA Degradation (If RNA-free genomic DNA is required, perform this optional step.)

◆ Add 5 µl of RNase A (50 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal

◆ Add **100 µl of TP Buffer** to the sample from **Step 2** and shake vigorously.

◆ Centrifuge at 14,000 x g for 3 minute. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

◆ Add 300 µl of absolute ethanol to the sample lysate and shake vigorously (break up any precipitate by pipetting but be careful to not let any pellet remain inside the pipette tip after you are done).

Step 4 DNA Binding

◆ Place a **TC Column** in a **2 ml Collection Tube**.

◆ **Transfer the mixture completely** from the previous step to the **TC Column**.

◆ Centrifuge at 14,000 x g for 30 seconds.

◆ Discard the flow-through and place the **TC Column** back in the **2 ml Collection Tube**.

Step 5 Wash

◆ Add **400 µl of W1 Buffer** into the **TC Column**. Centrifuge at 14,000 x g for 30 seconds.

◆ Discard the flow-through and place the **TC Column** back into the same **Collection tube**.

◆ Add **600 µl of W2 Buffer** (Ethanol added) into the **TC Column**. Centrifuge at 14,000 x g for 30 seconds.

◆ Discard the flow-through and place the **TC Column** back into the same **Collection tube.**

◆ Centrifuge at 14,000 x g again for 2 minutes to remove residual **W2 Buffer**.

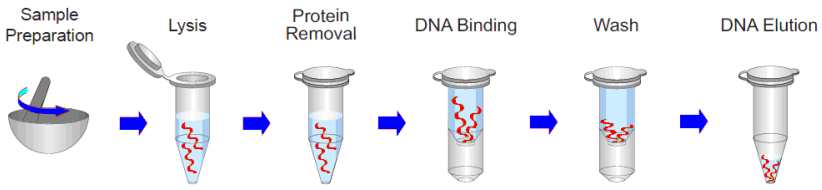
Step 6 DNA Elution

◆ Transfer the dried **TC 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 60ºC for 3 minutes.

◆ Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.



Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	Use 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 Buffer TL.

Low yield of DNA	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 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 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 containing 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.

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