Dual Genomic DNA Isolation Kit (Tissue)

Cat No. PDC06-0100
Size: 100 Reactions

Sample: 30~100 mg of fresh animal tissue
or 25 mg of paraffin-embedded
Tissue Format: Reagent and spin column
Operation time: within 1 hour
Elution volume: 50~200 µl

Description

The **Dual Genomic DNA Isolation Kit (Tissue)** combines reagent system and spin column system. The kit is designed specifically for genomic DNA isolation from animal tissue samples. This unique reagent system ensures the total DNA with high yield and good quality from samples. The spin column system is designed to purify or concentrate DNA products which have been previously isolated with the 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

Contents	PDC06-0100	PDC06-0100S
Buffer DG	100 ml	4 ml
Buffer BD	100 ml	4 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer E	10 ml	1 ml
Column DGT	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

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 tissue samples.
- Optimized tissue lysis buffer for the efficient lysis.

Application

- Gene cloning
- Southern blotting
- PCR
- SNP genotyping
- Quantitative real time PCR

Required Materials

- RNase A (50 mg/ml)
- Chloroform
- Isopropanol
- Liquid nitrogen
- Absolute ethanol
- Mortar and pestle
- 1.5ml microcentrifuge tubes

Quality Control

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

Dual Genomic DNA Isolation Kit (Tissue) Protocol

Step 1 Sample Preparation

Fresh Tissue

1. Cut off 100 mg of fresh animal tissue and grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.
2. Proceed with the Step2 Lysis.

Paraffin-embedded tissue

1. Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
2. Add 1 ml of xylene to the tube.
3. Vortex vigorously and incubate at room temperature for approximately 10 minutes.
4. Vortex occasionally during incubation.
5. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
6. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
7. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
8. Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
9. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
10. Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
11. Proceed with the Step2 Lysis.

Step 2 Lysis

1. Add 1 ml of Buffer DG 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.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate at 75°C for 30 minutes.(invert the tube every 10 minutes)
4. Centrifuge at 14-16,000 x g for 5 minutes.
5. Transfer carefully the clear supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Phase Separation

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

∗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

1. Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step3.
2. Mix the sample by inverting gently and let stand for 5 minutes at room temperature (DNA precipitation can be increased with extended standing time).
3. Centrifuge at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
5. 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.
6. Incubate for 10 minutes at 75ºC to dissolve the pellet.
7. If more pure DNA is required, perform this optional DNA Pure Protocol.

DNA Pure Protocol

#Add 60 ml of absolute ethanol to the Buffer W2 prior to initial use.
#Pre-heat the Buffer E to 75ºC prior to use.

Step 1 Sample Prep

1. Add 1 ml of Buffer BD to the sample which have been previously isolated using reagents and shake vigorously.

Step 2 Binding

1. Place a DGT Column in a Collection Tube. Apply the supernatant (from step 1) to the DGT Column by decanting or pipetting.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the DGT Column back into the same collection tube.

＊The maximum volume of the DGT Column reservoir is 800 µl. If the sample mixture is more than 800 µl, repeat the DNA Binding Step.

4. Centrifuge at 14-16,000 x g for 30 seconds.
5. Discard the flow-through and place the Column DGT back in the 2 ml Collection Tube.

Step 3 Wash

1. Add 400 µl of the Buffer W1 into the Column DGT
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the DGT Column back into the same collection tube.
4. Add 600 µl of the Buffer W2 (ethanol added) into the Column DGT
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the DGT 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 4 Elution

1. To elute the DNA, place the DGT Column in a clean 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer E or TE Buffer into the center of the column matrix.
3. Centrifuge at 14,000 x g for 2 minutes.

＊Check the buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

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	Increase the sample amounts prior to use. Grind tissue completely.
	Ethanol not added	Add the absolute ethanol to the Buffer W2 prior to the initial use. Make sure that the ethanol was added to the lysate before applying the sample to the Column DGP.
	Buffer E pH is too low	Check the pH
	Buffer E not pre-heated at 75°C	Pre-heat the Buffer E to 75°C prior to use.
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.
Inhibition of downstream Enzymatic reactions	Purified DNA containing residual ethanol	If the residual solution is seen in the purification column after washing the column with the 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 correct order for the Wash Buffers. Always wash the purification column with the Buffer W1 first, and then proceed to the wash with the Buffer W2

Caution

- Buffers B and W1 contain irritants. Wear gloves when handling these buffers.
- Add 60 ml of the ethanol to the Buffer W2 before use.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.