Dual Genomic DNA Isolation Kit (Tissue)

Cat. No.: SN019-0100 Size: 100 Reactions
Cat. No.: SN019-0004 Size: 4 Reactions
Sample: 30~100 mg of fresh animal tissue
Up to 25 mg of paraffin-embedded tissue
Format: Reagent and spin column
Operation time: within 60 minutes
Elution volume: 50~200 µl

Description

The Dual Genomic DNA Isolation Kit (Tissue) is designed to combine reagent system and spin column system. The kit could be used 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.

Features

- Ready-to-use genomic 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.

Applications

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

Kit Contents

Contents	SN019-0100	SN019-0004
Buffer DG	100 ml X 1 bottle	2 ml X 2 vials
Buffer BD	100 ml X 1 bottle	2 ml X 2 vials
Buffer W1	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (add ethanol)	15 ml X 1 bottle (60 ml X 1 bottle)	300 µl X 2 vials (1.2 ml X 2 vials)
Buffer BE	10 ml X 1 bottle	1 ml X 1 vial
DGT Column	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 2 bags	

Quality Control

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

Required Materials

- RNase A (50 mg/ml)
- Isopropanol
- Mortar and pestle
- Liquid nitrogen
- Chloroform
- Absolute ethanol
- Microcentrifuge tubes
- Water bath/ Dry bath

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 Step 2 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.
6. Remove the supernatant.
7. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
8. Centrifuge at 14-16,000 x g for 3 minutes.
9. Remove the supernatant.
10. Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
11. Centrifuge at 14-16,000 x g for 3 minutes.
12. Remove the supernatant.
13. Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
14. Proceed with the Step2 Lysis.

Step 2 Lysis

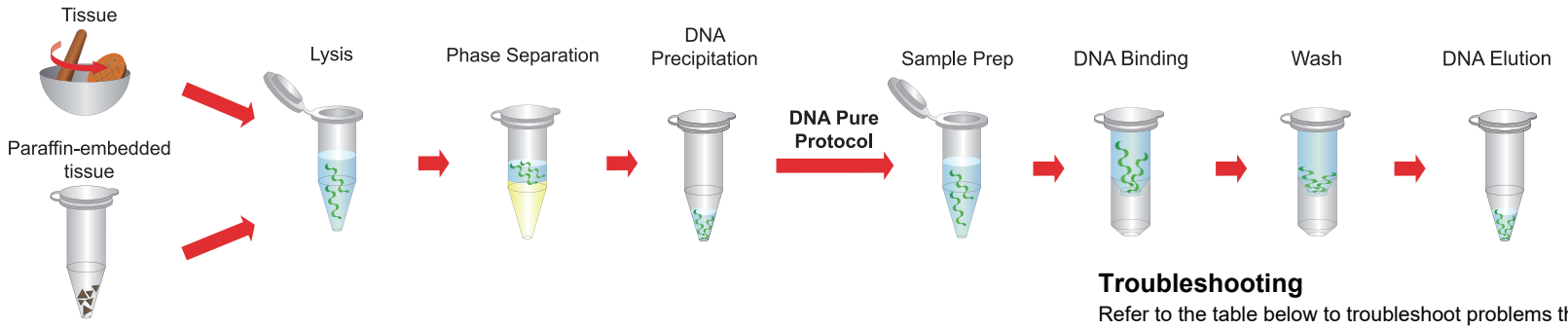
1. Add 1 ml of Buffer DG and 0.5 µl of RNase A (50 mg/ml) to the sample from Step 1 and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate at 75°C for 30 minutes.(invert the tube every 10 minutes)
4. Centrifuge at 14-16,000 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.
   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

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% ethanol.
5. Centrifuge at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 200 µl of TE buffer (not provided) or ddH_2O.
7. Incubate for 10 minutes at 75°C to dissolve the pellet.
8. If more pure DNA is required, perform this optional DNA Pure Protocol.



DNA Pure Protocol

#Pre-heat the Buffer BE to 75°C prior to use.

Step 1 Sample Prepareation

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

Step 2 DNA Binding

1. Place a DGT Column in a 2 ml Collection Tube.
2. Transfer the sample mixture from the previous step into the DGT Column.
3. Centrifuge at 14-16,000 x g for 30 seconds.
4. Discard the flow-through and transfer the remaining mixture into the same DGT Column.
5. Centrifuge at 14-16,000 x g for 30 seconds.
6. Discard the flow-through and place the DGT Column back in the 2 ml Collection Tube.

Step 3 Wash

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

Step 4 DNA Elution

1. Transfer the dried DGT Column to a new 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Pre-Heated Buffer BE or TE Buffer (not provided) into the center of the column matrix.
3. Let stand at 75ºC for 5 minutes.
4. Centrifuge for 2 minutes at 14-16,000 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	Grind tissue completely.
	Ethanol not added	Make sure that the ethanol was added to the lysate before applying the sample to the DGT Column.
	Buffer BE pH is too low	Check the pH
DNA degrade	Buffer BE not pre-heated at 75°C	Pre-heat the Buffer BE to 75°C prior to use.
	Sample is not fresh	1. Avoid repeated freeze / thaw cycles of the sample. 2. Use a new sample for the DNA isolation. 3. 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 minute at the maximum speed (≥12000 x g).
	Purified DNA contains residual salt.	1. Use the correct order for the Wash Buffers. 2. Always wash the purification column with the Buffer W1 first, and then proceed to the wash with the Buffer W2.

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and samples to prevent the DNase contamination.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up 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.