Instructions for use

Product Name: DTI Tissue DNA extraction kit

Cat# DT9765A

Description: The DTI Tissue DNA Extraction Kit is designed for DNA extraction and purification from variety of tissue samples from animal source. The kit employs a unique lysis buffer for tissue to release genomic DNA. Appropriate conditions for binding of DNA to the silica membrane spin columns is achieved by addition of ethanol to the lysate. Wash buffers provided in the kit efficiently remove contaminations yielding in highly purified genomic DNA.

The protocol provides a simple method to achieve the rapid isolation of highly purified genomic DNA and the entire procedure can be accomplished within 20 minutes after tissue cell lysis. Genomic DNA prepared by this kit is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular biology experiments.

Pack Size: 100 Preps

Contents:

Component	Quantity
Proteinase K (lyophilised)	1 vial
Proteinase K reconstitution buffer	1 vial
Buffer GL*1	24 ml
Buffer GB*1	24 ml
Buffer WA1 *1	56 ml
Buffer WB1 *2	48 ml
Elution buffer	28 ml
Spin columns	100 pcs
Collection tubes	100 pcs

*1 Contains strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.

*2 Before using the kit, add 112 ml of 100% ethanol. Mix well

Materials required but not provided:

1. 100% ethanol
2. Sterilized water
3. PBS
4. RNAse A (10 mg / mL) (as required)
5. Centrifuge
6. Vortex
7. Micropipettes
8. Water bath

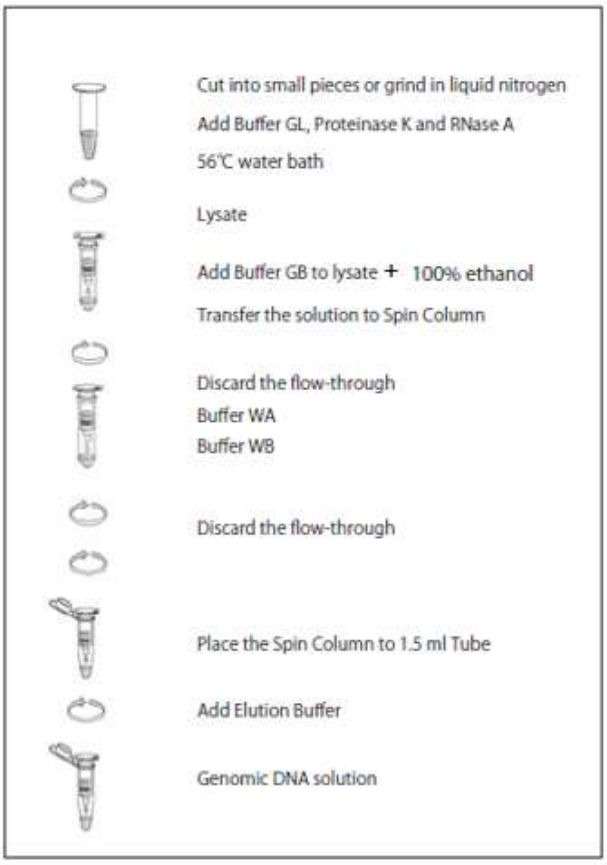
Storage and Shipment conditions:

1) The buffer contents can be stored at room temperature (15 - 25℃).
2) Recommended storage for Proteinase K and Proteinase K reconstitution buffer is 2-8℃ for 18 months.
3) Shipment temperature of the kit is room temperature (15 - 25℃)

Preparation before Experiment:

1. Proteinase K preparation: Dissolve the Proteinase K dry powder in the proteinase K reconstitution solution provided, and filter it with 0.22 um polyethersulfone (PES) or polyvinylidene fluoride (PVDF) membrane. Proteinase K (20mg/ml) is ready for use.
2. Adjust a water bath to 56℃.
3. If precipitation occurs in Buffer GL, warm at 65℃ and use after standing at room temperature.
4. Add 112 ml of 100% ethanol to Buffer WB and mix well before using it.
5. Pre-heat the Elution Buffer or sterile distilled water to 65℃ will improve elution efficiency.

Protocol at a glance:



Total working time post lysis of sample: ~ 20 mins

Protocol in detail is as below:

1. Homogenization and lysis: Each type of sample has different requirements for the method used to achieve efficient lysis and homogenization. Protocols are provided for the following types.

- For animal tissue:

i. Transfer 2 - 30 mg of animal tissue into 2 ml microtube. Cut into small pieces. Hard tissue can be grinded in liquid nitrogen.

ii. Add 180 µl of Buffer GL, 20 µl of Proteinase K and incubate it in 56℃ water bath with occasionally vortexing until the tissue has been completely lysed (2 - 3 hours). If the sample contains substances that are difficult to be lysed, it may take more time.

Note : If RNA-free DNA is crucial for downstream applications, 10 µl RNase A (10 mg / mL) solution may be added after addition of proteinase K.

iii. Add 200 µl of Buffer GB to the lysate and 200 µl 100% ethanol to the lysate and mix well**.**

- For aquatic organism such as fish (rich at small size DNA molecules)

i. Transfer 25 - 30 mg of tissue into 2 ml microtube. Cut into small pieces or grinded in liquid nitrogen.

ii. Add 180 µl of Buffer GL, 20 µl of Proteinase K and incubate it in 56℃ water bath until the tissue has been completely lysis (about 2 - 3 hours).
Note: The sample may occasionally be taken out from water bath and vortexed to accelerate tissue lysis.
If RNA-free DNA is crucial for downstream applications, 10 µl RNase A (10 mg / mL) solution may be added after addition of proteinase K.

iii. Add 200 µl of Buffer GB and 200 µl 100% ethanol to the lysate and mix well.

2. Transfer the solution to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.

3. Add 500 µl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

4. Add 500 µl of Buffer WB into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
Note : Make sure the amount of 100% ethanol specified on the bottle label has been added to Buffer WB.
Add Buffer WB along the wall of Spin Column to wash off any residual salt.

5. Repeat Step 4.

6. Place Spin Column into Collection Tube. Centrifuge at 12,000 rpm for 2 minutes. This step is to ensure complete removal of residual ethanol.

7. Place Spin Column into a new 1.5 ml microtube. Add 50 - 200 µl of Elution Buffer or sterile distilled water to the centre of the Spin Column membrane. Let it stand for 5 minutes at room temperature.
Note : Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve recovery efficiency.

8. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
9. Store the DNA at -20°C for further analysis.

Note: This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals.