DTI FabSpin Universal DNA extraction kit

Instruction Manual for use

User Manual for DNA extraction from various sample types

Catalogue number: DT9701.25P/DT9701.100P/DT9701.250P

Kit for Research use purpose only

Manufactured by
DSS Takara Bio India Pvt Ltd

Table of Contents

I. Representative Quick reference protocol for extraction of DNA from animal tissue

Cut 25-30 mg tissue into small pieces or grind in liquid nitrogen
Add 180 µl Buffer GL, 20 µl Proteinase K and incubate in
56°C water bath for 2-3 hrs until the tissue has completely lysed

Tissue lysis

Add 200 µl Buffer GB and 200 µl 100% ethanol and mix well.

Transfer the solution to spin column.

DNA precipitation and binding

Centrifuge at 12,000 rpm for 2 mins
Discard the flow through.

Add 500 µl of Buffer WA

Washing

Centrifuge at 12,000 rpm for 1 min
Discard the flow through.

Add 500 µl of Buffer WB

Centrifuge at 12,000 rpm for 1 min
Discard the flow through.

Place the spin column to 1.5 ml tube

Centrifuge at 12,000 rpm for 2 mins to remove residual salts

Add 50 µl of Elution Buffer.
Allow to stand at RT for 5 mins
Centrifuge at 12,000 rpm for 2 mins
Store Genomic DNA at -20°C

DNA elution

Note:

i) *Lysis step depends on the sample type. Refer the Homogenization and lysis section for details*

ii) *During any stage if the contents in the tube touches the cap, perform a brief spin step to bring the contents down, thereby reducing risk of contamination*

2) Kit Contents:

Component	Cat# DT9701.25P (25 Preps)*	Cat# DT9701.100P (100 Preps)	Cat# DT9701.250P (250 Preps)
Proteinase K (lyophilised)	10 mg	40 mg	100 mg
Proteinase K reconstitution buffer	500 µl	2 ml	5 ml
Buffer GL **	6 ml	24 ml	60 ml
Buffer GB **	6 ml	24 ml	60 ml
Buffer WA **	14 ml	56 ml	140 ml
Buffer WB	12 ml	48 ml	120 ml
Elution buffer	7 ml	28 ml	70 ml
PBS	5 ml	20 ml	50 ml
Nuclease free water	1.25 ml	5 ml	12.5 ml
Spin columns (Green ring)	25 pcs	100 pcs	250 pcs
Collection tubes	25 pcs	100 pcs	250 pcs
SDS insert sheet	1	1	1
CoA	1	1	1

*Sample Pack, available only on request
**Contains strong denaturant. Avoid contact with skin and eyes. In case of such contact, wash immediately with plenty of water and seek medical advice. Please refer the safety data sheet

3) Materials required but not provided:

- Sterile mortar and pestle
- Liquid Nitrogen
- 100% ethanol
- Sterilized water
- Phosphate Buffered Saline (PBS)
- RNAse A (10 mg / mL) (as required)
- Centrifuge
- Vortex
- Water bath (56°C)
- Micropipettes (2-200 µl, 10-1000 µl)
- Pipette tips (200 µl, 1000 µl)
- Personnel protection equipment including labcoat, gloves and goggles

4) Storage and shipment conditions:

- The buffer components are to be stored at room temperature (15 - 25°C).
- Lyophilised powder preparations of Proteinase K to be stored at -20°C for 2 years/ 4°C for 18 months/ RT for 12 months. Once reconstituted, store at -20°C.
- Shipment temperature of the kit is room temperature (15 - 25°C).

5) Product Description:

Principle:

The DTI FabSpin Universal DNA extraction Kit is designed for DNA extraction and purification from a variety of from a variety of sample sources including blood, gram-negative bacteria, mammalian cultured cells, plant tissue and animal tissue. This kit employs a proprietary lysis buffer in combination with Spin Column membrane to efficiently purify genomic DNA from the biological sample. 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 lysed. Using the kit about 10 µg of highly purified genomic DNA can be extracted from 50 - 200 µl of mammalian whole blood (with anticoagulant), 1 - 10 µl of nucleated erythrocyte in whole blood (with anticoagulant), 1.0 - 5.0 x 10^9 gram-negative bacteria, 1.0 x 10^5 - 1.0 x 10^7 cultured cells, 2 - 30 mg of mammalian tissues and 25 - 100 mg plant tissues. 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.

Kit specifications:

- The DTI FabSpin Universal DNA extraction Kit is designed for rapid isolation of high purity DNA from variety of sample sources including blood, gram-negative bacteria, mammalian cultured cells, plant tissue and animal tissue
- High pure genomic DNA with an A_{260}/A_{280} ratio between 1.8-2.0 can be obtained using this kit
- Genomic DNA obtained is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular experiments.

6) Preparation before experiment

i. Proteinase K preparation:

For Cat# DT9701.25P, dissolve 10 mg of lyophilised Proteinase K in 500 µl of reconstitution buffer provided in the kit.

For Cat# DT9701.100P, dissolve 40 mg of lyophilised Proteinase K in 2 ml of reconstitution buffer provided in the kit.

For Cat# DT9701.250P, dissolve 100 mg of lyophilised Proteinase K in 5 ml of reconstitution buffer provided in the kit.

ii. If precipitation occurs in Buffer GL, warm at 65℃ and use after standing at room temperature.

iii. Adjust a water bath to 56°C.

iv. Before using the kit:

For Cat# DT9701.25P, add 28 ml of 100% ethanol to Buffer WB (12 ml). Mix well.

For Cat# DT9701.100P, add 112 ml of 100% ethanol to Buffer WB (48 ml). Mix well.

For Cat# DT9701.250P, add 280 ml of 100% ethanol to Buffer WB (120 ml). Mix well.

v. Pre-heat the Elution Buffer to 65°C to improve elution efficiency.

7) Protocol in detail

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 each sample type.

For animal and plant tissue:

(1) Transfer 2 - 30 mg of animal tissue or 25 - 100 mg of plant tissue into 2 ml microtube. Cut into small pieces. Hard tissue can be grinded in liquid nitrogen.

(2) 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 lysis. (2 - 3 hours. If there is a materials difficult for lysis, it may take more time.

Note : Some plant samples contain fibrous tissue which is difficult to lysis. This kit, however, is available for these samples also.

(3) Add 200 µl of Buffer GB to the lysate and mix well

For whole blood:

(1) Transfer 1 - 10 µl of nucleated erythrocytes in whole blood (with anticoagulant), 50 - 200 µl of anucleated erythrocytes in whole blood (with anticoagulant) into 2 ml microtube. Add PBS or sterilized water up to 200 µl.

(2) Add 180 µl of Buffer GB and 20 µl of Proteinase K. Mix well and incubate it in 56℃ water bath for 10 minutes.

For mammalian cultured cells grown in suspension:

(1) Transfer 1.0 x 10^5 - 1.0 x 10^7 cell suspension in 1.5 ml microtube. Centrifuge at 5,000 rpm for 5 minutes. Discard the supernatant.

(2) Add 200 µl of PBS or sterilized water to resuspend the cells.

(3) Add 180 µl of Buffer GB and 20 µl of Proteinase K. Mix well and incubate it in 56℃ water bath for 10 minutes.

For mammalian adherent cells:

(1) Discard the supernatant as much as possible. Add 1 ml of PBS to each 10 cm2 of Adherent cells. Pipette up and down several times to pull off the adherent cells, and then transfer the cell suspension to 1.5 ml microtube. Centrifuge at 5,000 rpm for 5 minutes. Discard the supernatant. Add 200 µl of PBS or sterilized water to resuspend the cells.

(2) Add 180 µl of Buffer GB, 20 µl of Proteinase K. Incubate it in 56℃ water bath for 10 minutes.

For gram-negative bacteria such as E. coli:

(1) Transfer 1.0 - 5.0 x 10^9 of bacteria into 1.5 ml microtube. Centrifuge at 12,000 rpm for 2 minutes. Discard the supernatant.

(2) Add 180 µl of Buffer GL, 20 µl of Proteinase K. Mix well and incubate it in 56℃ water bath for 10 minutes.

(3) Add 200 µl of Buffer GB. Mix well.

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

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

(2) 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.
(3) Add 200 µl of Buffer GB and 200 µl 100% ethanol to the lysate and mix well.
For a processed product (rich at fragmented genomic DNA)
(1) Transfer 25 - 50 mg of a processed product into 2 ml microtube. Cut into small pieces.or grinded in liquid nitrogen.
(2) 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.
(3) Add 200 µl of Buffer GB to the lysate and mix well

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.

2. DNA precipitation and binding:

i. Add 200 µl 100% ethanol and mix well.
ii. Transfer the mixture to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.

3. Wash silica membrane:

i. 1st wash: Add 500 µl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
ii. 2nd wash: 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 the Buffer WB. Add Buffer WB along the tube wall of Spin Column to wash off any residual salt.
iii. Repeat 2nd wash.
iv. Place Spin Column in Collection Tube. Centrifuge at 12,000 rpm for 2 minutes. This step is to ensure complete removal of residual ethanol.

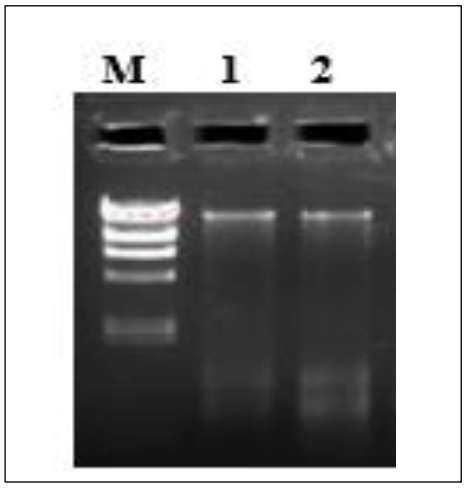
4. Elution:

i. Place Spin Column in a new 1.5 ml tube. Add 50 µl of Elution Buffer to the centre of the membrane. Let it stand for 5 minutes at room temperature.
ii. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
iii. 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.

7) Experimental sample:

About 3 µg of high-purity genomic DNA was extracted from 25 mg of fish gill tissue is obtained using the DTI FabSpin universal DNA extraction kit. Below is the electrophoresis image of genomic DNA.



Lane M: λ Hind III digest marker
Lane 1-2: Test in multiples

8) DNA Extraction from Various Materials:

The yields of DNA extracted are shown as follows.

Material	Tissue amount	DNA yield
Mouse Liver	25 mg	15 - 20 µg
Mouse Spleen	5 - 10 mg	20 - 40 µg
Mouse Tail	1.2 cm	10 - 15 µg
Mouse Brain	25 mg	10 - 20 µg
Mouse Kidney	25 mg	15 - 30 µg
Mouse Lung	25 mg	15 - 30 µg
Mouse Intestine	25 mg	15 - 30 µg
Mouse Ear	25 mg	10 - 20 µg
Carp Fin	25 mg	10 - 20 µg
Short Necked Clam Meat	25 mg	3 - 10 µg
HL60 Cultured Cells	2.0 x 106	10 - 15 µg
E. coli JM109	2.0 x 109	5 - 10 µg
Horse Blood	200 µl	2 - 5 µg
Fish Blood	5 µl	10 µg
Celery	25 mg	4 - 6 µg

Rape	25 mg	1 - 2 µg
Spinach	25 mg	2 - 3 µg

9) Maximum Amount of Starting Materials:

Material	Maximum amount	Recommended amount
Cultured Cells	1.0 x 10^7	2.0 x 10^6
Nucleated erythrocytes in whole blood	10 µl	1 ～ 5 µl
Anucleated erythrocytes in whole blood	200 µl	50 ～ 200 µl
E. coli and other gram-negative bacteria	5.0 x 10^9	2.0 x 10^9
Common animal tissue	30 mg	25 mg
Tissue of high DNA content (e.g. spleen)	10 mg	5 mg
Tissue of higher DNA content (e.g. calf thymus)	2 mg	1 mg
Plant material	100 mg	25 ～ 50 mg

10) Q & A:

Q1. What is the extraction yield of genomic DNA?

A1. This kit is suitable for purification of genomic DNA from plant, whole blood, cultured cells and gram-negative bacteria. The yield of genomic DNA differs by starting material. Generally, 15 µg of genomic DNA can be extracted from 25 mg of liver tissue; 10 µg of genomic DNA can be extracted from 100 mg of spinach; 0.5 µg of genomic DNA can be extracted from 100 µl of human whole blood; 10 µg of genomic DNA can be extracted from 2.0 x 10^6 of cultured cells; 10 µg of genomic DNA can be extracted from 2.0 x 10^9 of E. coli cells.

Q2. The yield of genomic DNA is low or there is no yield, why?

A2. When the yield of genomic DNA is lower, the following aspects can be considered:

(1) The experimental material is not enough, for instance, genomic DNA from 2 x 10^3 cultured cells can't be detected by electrophoresis.

(2) Incomplete grinding animal tissue or plant tissue causes incomplete release of DNA. It's recommended to extending the lysis time (up to overnight) or increasing the amount of lysis buffer.

(3) The content of genomic DNA in material is low. Increase the amount of starting material.

(4) The amount of starting material is too much so it's hard for lysis. Increase the amount of lysis buffer appropriately or divide the material into more than one.

(5) Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.

(6) Strictly follow the protocol.

Q3. Why is the extracted genomic DNA degraded?

A3. (1) The experimental materials are not fresh enough or the tissue materials are not processed in time or not stored at low temperature after collecting. It is recommended that the materials should be stored at -80℃ and shipped using dry ice.
(2) There is residue DNase on experimental materials. Wash with Buffer WA once more.

Q4. Why is there contamination with RNA in extracted genomic DNA?

A4. (1) RNase A has not been used in operation. Strictly follow the protocol to use RNase A.
(2) RNase A may be inactivated. RNase A should be stored at -20℃. RNase A is stable and not easily inactivated.

Q5. Why does the extracted genomic DNA have low biological activity?

A5. (1) The salt concentration in extracted genomic DNA is too high. When washing the Spin Column membrane using Buffer WA and Buffer WB, add them along the tube wall of Spin Column and let it stand for 5 minutes at room temperature to wash off any residual salt and improve the washing effect.

(2) There is residual ethanol in lysate. Let the column stand for 2 minutes at room temperature before adding lysis solution to the column and it will improve the effect of washing.

(3) The elution buffer must be added in the centre of Spin Column membrane at DNA elution and not residue on the tube wall of Spin Column.

Q6. How to extract when amount of materials is more than the amount in the protocol?

A6. This kit is designed to purify a small amount of genomic DNA. When the amount of materials is more than the amount described in the protocol, increase the Buffer GL or Buffer GB and divide the obtained homogenate into two tubes. It is important to adjust the amount of starting material in the specified range. The incomplete lysis will block the Spin Column and cause the failure of DNA purification.

Q7. How to extract genomic DNA from aquatic such as fish?

A7. The standard protocol of this kit is adapted to extract large size of genomic DNA. Most genomic DNA of fish and other aquatic animals is, however, smaller size genomic DNA than that of mammals. Then you should follow the protocol of “For lysis of aquatic organism such as fish” for homogenization and lysis.

Q8. How to extract genomic DNA from a processed product?

A8. ・The standard protocol of this kit is adapted to extract large size genomic DNA. Most genomic DNA of a processed product is degraded into small size DNA in the process. Then you should follow the protocol of “For a processed product” for homogenization and lysis.

・A processed product is low DNA content and its DNA is degraded during processed. The DNA from a processed product cannot be detected as bands by gel electrophoresis.

12) Ordering information:

Catalogue No.	Product Description	Pack Size
DT9701.250P	DTI FabSpin Universal DNA extraction kit	250 reactions
DT9701.100P	DTI FabSpin Universal DNA extraction kit	100 reactions

Visit www.dsstakarabio.com/DTI for more detailed product information

For more information contact directly below;
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Email: enquiries@dsstakarabio.com
Toll-Free number 1800-212-4922

Description of Symbol Used:

REF Catalogue number

LOT Batch Code

Date of Manufacturing

Use-by-date

Contains sufficient for <n> tests