



DTi FabSpin Blood DNA Kit II

Instruction Manual for use



User Manual for DNA extraction from blood samples

Catalogue number: DT9702.100P/ DT9702.250P











Kit for Research use purpose only

Manufactured by
DSS Takara Bio India Pvt Ltd

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Genomic DNA from blood Quick reference protocol

	Add 200 µl Buffer GB, 20 µl Proteinase K, and 200 µl whole blood* Incubate in 56°C Water bath for 10 mins.	Cell lysis
	Add 200 µl 100% ethanol and mix well.	
	Transfer the solution to green ring spin column.	DNA precipitation and binding
	Centrifuge at 12,000 rpm for 2 mins Discard the flow through.	
	Add 500 µl of Buffer WA	
	Centrifuge at 12,000 rpm for 1 min Discard the flow through.	Washing
	Add 700 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

*Refer Protocol Section1, note b) in case the starting material is < 200 µl

2) Kit Contents:

Component	Cat# DT9702.25P (25 Preps)*	Cat# DT9702.100P (100 Preps)	Cat# DT9702.250P (250 Preps)
Proteinase K (lyophilised)	10 mg	40 mg	100 mg
Proteinase K reconstitution buffer	500 µl	2 ml	5 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
Instruction manual	±	±	±
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:

- 100% ethanol
- 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
- Proteinase-K to be purchased separately for catalogue numbers DT9702.25/
DT9702.100/ DT9702.250

4) Storage and shipment conditions:

- The buffer components are to be stored at room temperature (15 - 25°C).
- Once received, store the Lyophilised powder preparations of Proteinase K at -20°C
- Shipment temperature of the kit is room temperature (15 - 25°C).

5) Product Description:

Principle:

The DTI FabSpin Blood DNA Kit II is designed to purify genomic DNA from whole blood. 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 30 mins. Genomic DNA obtained is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular experiments.

Kit specifications:

- The DTI FabSpin Blood DNA Kit II is designed for rapid isolation of high purity DNA from whole blood of higher mammals including humans with non-nucleated Red blood cells RBCs
 - This kit can also be used for extraction of genomic DNA from birds and fish containing nucleated RBCs
- DNA can be purified successfully from blood samples collected in EDTA, ACD or heparin anti-coagulant. DNA can also be purified from buffy coat of human blood sample
- High pure genomic DNA with an A_{260}/A_{280} ratio between 1.8-2.0 with a concentration of 3-5 μg from healthy individual 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# **DT9702.25P**, dissolve 10 mg of lyophilised Proteinase K in 500 μl of reconstitution buffer provided in the kit.

For Cat# **DT9702.100P**, dissolve 40 mg of lyophilised Proteinase K in 2 ml of reconstitution buffer provided in the kit.

For Cat# **DT9702.250P**, dissolve 100 mg of lyophilised Proteinase K in 5 ml of reconstitution buffer provided in the kit.

ii. Adjust a water bath to 56°C.

iii. Before using the kit:

For Cat# **DT9702.25P**, add 28 ml of 100% ethanol to Buffer WB (12 ml). Mix well.

For Cat# **DT9702.100P**, add 112 ml of 100% ethanol to Buffer WB (48 ml). Mix well.

For Cat# **DT9702.250P**, add 280 ml of 100% ethanol to Buffer WB (120 ml). Mix well.

iv. Pre-heat the Elution Buffer/nuclease free water to 65°C to improve elution efficiency.

7) Protocol in detail

I) Purification of DNA from whole blood with non-nucleated RBCs e.g., mammals including humans is as below

1. Cell lysis:

- i. For higher mammals with anucleated RBCs : Add 200 µl of Buffer GB, 20 µl of Proteinase K (20 mg/ml) in an empty 1.5 ml microcentrifuge tube. Add 200 µl of whole blood to this, Mix well and incubate it in 56°C water bath for 10 minutes.
- ii. For extraction of genomic DNA from birds and fish containing nucleated RBCs: Use not more than 10 µl of blood for the extraction process. Make up the volume to 200 µl using PBS provided with the kit. Add this to the 200 µl of Buffer GB, 20 µl of Proteinase K (20 mg/ml) and proceed with next step

Note:

- a) 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.
- b) For higher mammals if the volume of blood available is <200 µl, the extraction can be performed by making up the 200 µl using PBS provided with the kit.

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 700 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 or nuclease free water (as appropriate) 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:** a) Fresh blood collected in EDTA/ACD anti-coagulant is preferred for molecular testing including PCR.
- b) Fresh blood collected in Heparin anti-coagulant is preferred for Genetic testing including Karyotyping, FISH.

5) Troubleshooting

1. Low/no yield of DNA is obtained.
 - i. Pre-heat the Elution Buffer at 65°C can improve elution efficiency.
 - ii. The starting amount is lower or whole blood is not fresh enough (ensure to avoid repeated freeze-thawing).
 - iii. Use buffy coat as the starting material. This can be prepared by centrifuging the whole blood at 2000 rpm for 5 mins. The buffy coat layer will be visible between the RBCs and the plasma layer. Aspirate the buffy coat and proceed the extraction from the WBC lysis step.
2. The extracted genomic DNA is degraded.
 - i. The whole blood is not fresh enough. Blood samples with anticoagulant can be stored for 10 days at 2 - 8°C.
3. RNA contamination in extracted genomic DNA.
 - i. Follow the protocol to use RNase A (10 mg/ml).

4. Extracted genomic DNA of poor quality.
- i. Salt concentration in extracted genomic DNA is too high. When washing the DNA preparation membrane with Buffer WA and Buffer WB, add these along the tube wall of Spin Column and stand for 5 minutes at room temperature to wash off any residual salt.
 - ii. There is residual ethanol in genomic DNA solution. Stand the Spin Column for 2 minutes at room temperature before adding Elution Buffer to the Spin Column and it will improve the effect of elution.
 - iii. The Elution Buffer must be added in the center of membrane during DNA elution and not along tube wall of Spin Column.
 - iv. Filter sterilise the proteinase K solution prepared using 0.22 um polyethersulfone (PES) or polyvinylidene fluoride (PVDF) membrane

6) Ordering information:

Catalogue No.	Product Description	Pack Size
DT9702.250P	DTI FabSpin Blood DNA kit with Proteinase K included in the kit	250 reactions
DT9702.100P	DTI FabSpin Blood DNA kit with Proteinase K included in the kit	100 reactions

Visit <https://dsstakarabio.com/pages/dti-fabspin-blood-dna-kit-ii> 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:

-  Catalogue number
-  Batch Code
-  Date of Manufacturing
-  Use-by-date
-  Contains sufficient for <n> tests

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and cannot be seen as a kind of recommendation or assessment). Regarding these products or services, we cannot grant any guarantees regarding selection, efficiency, or operation.