Instructions for use

Product Name: DTI FabSpin Blood DNA Kit

Cat# DT9781A

Description: The DTI FabSpin Blood DNA Kit is designed to purify genomic DNA from whole blood of mammals with non-nucleated erythrocyte. This kit may also be used for extraction of genomic DNA from birds and fish containing nucleated erythrocyte.

The kit employs a unique lysis buffer for non-nucleated erythrocyte and leukocytes to release genomic DNA. Appropriate conditions for binding of DNA to the silica membrane spin columns are achieved by addition of ethanol to the lysate. Wash buffers provided in the kit efficiently remove contaminations yielding in highly purified genomic DNA. The entire procedure can be accomplished within 1 hour. Genomic DNA obtained is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular experiments.

Pack Size: 100 Preps

Contents:

Component	Quantity
10X Buffer RCL A	8 ml
10X Buffer RCL B	32 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. 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.

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

Materials required but not provided:

1. Proteinase K (20mg/ml)
2. 100% ethanol
3. Sterilized water
4. PBS
5. RNAse A (10 mg / mL) (as required)
6. Centrifuge
7. Vortex
8. Micropipettes
9. Water bath

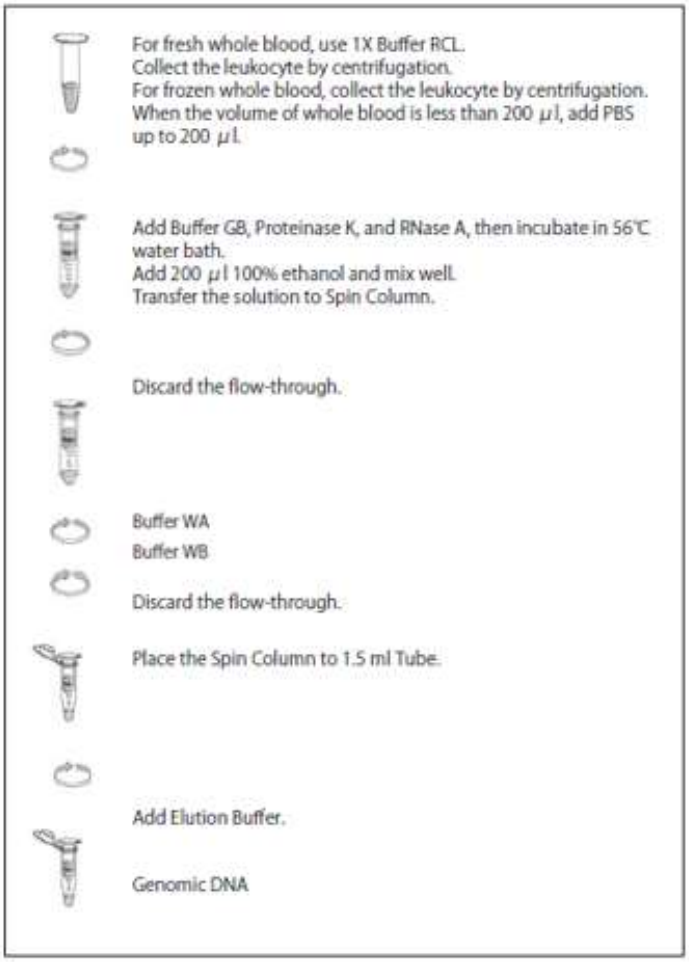
Storage and Shipment conditions:

1) The buffer contents are to be stored at room temperature (15 - 25℃).
2) 10X Buffer RCL A and 10X Buffer RCL B can be stored at room temperature. The mixed 10X Buffer RCL can be stored at 4℃ for 1 year.
3) Shipment temperature of the kit is room temperature (15 - 25℃)

Preparation before Experiment:

1. Adjust a water bath to 56℃.
2. Add 112 ml of 100% ethanol to Buffer WB and mix well before using it.
3. Pre-heat the Elution Buffer or sterile distilled water to 65℃ will improve elution efficiency.

Protocol at a glance:



Lysis: Total incubation for 25 mins + 7 mins centrifugation

Precipitation and binding of DNA to columns: 2 mins centrifugation

Washing : 5 mins centrifugation (1 min/wash + additional 2 mins to remove residual ethanol

Elution : 5 min incubation + 2 min centrifugation

Total working time ~55-60 mins

Protocol in detail is as below:

Sample preparation

1. For whole blood with non-nucleated erythrocyte:
    - 200 µl - 1 ml fresh whole blood: process sample from steps 1 - 15 given below.
    - 200 µl - 1 ml frozen whole blood: centrifuge at 2,000 rpm for 5 minutes, then 200 µl of the supernatant and precipitate is to be used, process sample from steps 7 – 15 given below.

    For whole blood less than 200 µl, add PBS to make the volume to 200 µl and process from steps 7 – 15 given below.

2. For whole blood with nucleated erythrocyte:

Do not use more than 10 µl of whole blood. Add PBS up to make the volume to 200 µl and proceed from steps 7 – 15 given below.

Note: Fresh blood collected in EDTA anti-coagulant is preferred for molecular testing

DNA Extraction

1. Prepare 1X Buffer RCL - First, mix 10X Buffer RCL A and 10X Buffer RCL B in the ratio of 1:4 to obtain 10X Buffer RCL. Then add sterilized water 9 times the volume of the 10X Buffer RCL to prepare 1X Buffer RCL. Prepare 1X Buffer RCL twice the volume of whole blood.
   Note: 1X Buffer RCL should be freshly prepared before use.
2. Add 1.5 volume of 1X Buffer RCL in the whole blood sample and mix well. Allow to stand for 15 minutes at room temperature (15 - 25℃).
3. Centrifuge at 2,000 rpm for 5 minutes (Do not use greater centrifugal force). Discard the supernatant by decantation.
4. Add 1X Buffer RCL of 0.5 volume of the whole blood sample to the precipitate and mix well. Allow to stand at room temperature for 10 minutes.
5. Centrifuge at 2,000 rpm for 2 minutes (Do not use greater centrifugal force). Discard the supernatant carefully. Check whether the precipitate is red in color due to presence of erythrocyte. If red color observed, repeat Step 4 until there is no red color observed in the precipitate. (In most mammalian whole blood, red color can be cleared by 2 times treatment, but blood with red cell having thicker membrane such as horse blood can require more times treatment).
6. Add 200 µl PBS in the precipitate to resuspend cells.
7. Add 200 µl of Buffer GB, 20 µl of Proteinase K (20 mg/ml), Mix well and incubate it in 56℃ water bath for 10 minutes.
   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.
8. Add 200 µl 100% ethanol and mix well.
9. Transfer the mixture to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.
10. Add 500 µl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
11. 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.
12. Repeat Step 11.

13. Place Spin Column in Collection Tube. Centrifuge at 12,000 rpm for 2 minutes. This step is to ensure complete removal of residual ethanol.
14. Place Spin Column in a new 1.5 ml tube. Add 30 - 200 µl of Elution Buffer or sterile distilled water to the centre of the membrane. Let it stand for 5 minutes at room temperature.
    Note: Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.
15. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
16. Store the DNA at -20℃ 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.

Description of Symbol Used:

REF Catalogue number

LOT Batch Code

Date of Manufacturing

Use-by-date

Contains sufficient for <n> tests

Upper limit of temperature

Manufacturer