MQ Blood Genomic DNA Extraction Kit

Cat No. BGE-M-002-100
(100 Preps)

MOLEQULE-ON®

Component	100 Preps BGE-M-002-100
Red Blood Cell Lysis Buffer	120ml
Leukocyte Lysis Solution A	50ml
Protein Precipitate Solution B	50ml
Washing Buffer*	15ml x 2
Elution Buffer	20ml
Proteinase K	1ml x 2
RNase A	100ul x 2
MQ Columns and 2ml Collection Tubes	100 Each

Preparations

* Note: Please add 45ml of Absolute Ethanol to the 15ml of Washing Buffer before use.
Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

Storage

This kit should be stored dry at 15˚C - 25˚C. The proteinase K and RNase A included in the kit should be stored at – 20˚C.

Description

MQ Blood Genomic DNA Extraction Kit uses a centrifugal adsorption column that can specifically bind DNA and a unique buffer system to extract genomic DNA from whole blood. The silicon matrix material used in the centrifugal adsorption column is a unique new material, which can efficiently and specifically adsorb DNA, and can remove impurity proteins and other organic compounds in cells to the greatest extent. The extracted genomic DNA fragments are large, high in purity, stable and reliable in quality. The genomic DNA extracted by this kit can be used in various routine operations, including restriction enzyme digestion, PCR, library construction, Southern hybridization and other experiments.

Features

- Fast and easy processing using a rapid spin-column format. The entire procedure takes approximately 30 minutes.
- High quality of DNA. $OD_{260}/_{280}$ of purified DNA is generally 1.7-1.9.
- No phenol/chloroform extraction or ethanol precipitation is required.

Materials Supplied by User

Microcentrifuge capable of at least 12,000 × g, Micropipettes and pipette tips, Vortexer, Ethanol (96-100%), Microcentrifuge tubes (1.5 ml or 2 ml), Water bath for heating at 56°C

Preparation of Blood Samples

1. Preparation of blood samples (This kit is designed for 100-500ul fresh or anticoagulant-added blood sample)

a. Add double volumes of Red Blood Cell Lysis Buffer, mix thoroughly by inverting up and down, incubate at room temperature for 2-5 minutes, centrifuge at 12000rpm for 2 minutes, carefully aspirate the supernatant, the precipitate should be white or light red. If the lysis is not complete, you can repeat the above steps once.

b. If the sample is blood from poultry, birds, amphibians, of which red blood cells have nucleolus, the capacity of blood sample should be reduced to 5-20 µl and lysis of erythrocytes by Red Blood Cell Lysis Buffer is not required. Add 500µl solution A directly, mix thoroughly.

Procedure

2. Add 500ul of Solution A, mix with a pipette tip by sucking up and down until mix thoroughly. Incubate in water bath at 65°C for 10 minutes. Invert mix the tube several time during incubation until the solution is clear without obvious cells.

3. After the tube temperature drops to room temperature, add 2µl RNase A (100mg/ml) and 20ul of Proteinase K(10mg/ml), mix thoroughly by inverting the tube, incubate at room temperature for 10min.

4. Add 500µl Protein Precipitate solution B, mix thoroughly by inverting the tube. If turbidity occurs, incubate in 60°C water bath for 10 min. Centrifuge the tube at 12000 rpm for 5 minutes at 4°C. Carefully aspirate the lower liquid with a micropipette (do not suck the upper layer or floating material), transfer the liquid to a clean 1.5ml microcentrifuge tube, if there is sediment, centrifuge again.

5. Add 0.7 volume absolute ethanol, mix thoroughly by inverting the tube, flocculent precipitation may occur, which will not affect the extraction of DNA. Solution and precipitation both can be added to the MQ Adsorption Column and incubate at room temperature for 2 minutes.

6. Centrifuge at 12000rpm for 1 min at 4°C, discard the flow-through, and re-use the collection tube in the next step.

7. Wash the MQ Adsorption Column with 600µl Washing Buffer (ensure that absolute ethanol has been added), centrifuge at 12,000rpm for 1 min at 4°C, discard the flow-through and re-use the collection tube in the next step.

8. Repeat step 7 with another 600µl Washing buffer.

9. Centrifuge at 12,000rpm for 2min. Allow the column to air dry with the cap open for 2 minutes to dry the membrane at room temperature or 50℃. It is critical for removing ethanol from the column. Otherwise, ethanol in Washing Buffer will affect subsequent experiments such as enzyme digestion and PCR.

10. Place column into a new clean 1.5ml microcentrifuge tube. Add 50-100µl Elution buffer which is preheated by 65℃ water bath to the center of silica membrane matrix, incubate at room temperature for 5min and centrifuge at 12,000rpm for 2min.

11. To increase DNA concentration, add the solution obtained from step 10 to the center of membrane again, centrifuge at 12,000 rpm for 2 min.

Notes

1. The most common of anticoagulant include: EDTA, ACD, heparin, if need big size Blood Genomic DNA, ACE may be better.
2. Avoid repeated freezing and thawing of samples. Otherwise, the extracted DNA fragments are smaller and the extracted amount is also decreased.
3. If the precipitate occurs in the kit components, re-dissolved in 65℃ water bath before use, which will not affect the results.
4. The erythrocytes in the whole blood of most mammals are non-nucleated, so the non-nucleated erythrocytes without DNA should be removed when extracting genomic DNA. If the sample is blood from poultry, birds, amphibians or lower grade lives, of which erythrocytes have nucleolus, the amount should be reduced to 5-20µl and lysis of erythrocytes by Red Blood Cell Lysis Buffer is not required.
5. If the volume of elution buffer is less than 50µl, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0 (adjusted by NaOH), below 7.0 will affect elution efficiency.