Instructions for Use

Version: 1.0.2

Blood Genomic DNA Kit

Catalog No.: abx098078

Size: 50 rxns / 200 rxns

Storage: Store RNase A at -20 °C for up to two years, and the other components at room temperature (15-25 °C) for up to one year.

Introduction

Abbexa's Blood Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from 5-250 µl of fresh or frozen blood. Binding buffer is added to blood to release DNA, which is then isolated using a silica-based column. The isolated genomic DNA is suitable for PCR, restriction enzyme digestion and southern blotting.

Kit Components

Reagent	50 rxns	200 rxns
Binding Buffer	30 ml	110 ml
Clean Buffer	6 ml	24 ml
Wash Buffer	12 ml	2 × 22 ml
Elution Buffer	25 ml	80 ml
RNase A (10 mg/ml)	500 µl	2 × 1 ml
Proteinase K (20 mg/ml)	1 ml	4 × 1 ml
Genomic Spin Columns with Collection Tubes	50	200

Protocol

Reagent Preparation

- **Working Clean Buffer solution:** Dilute the Clean Buffer with 96-100% ethanol to a ratio of 1:4 (i.e. add 6 ml of Clean Buffer into 24 ml of 96-100% ethanol to form 12 ml of Working Clean Buffer solution).
- **Working Wash Buffer solution:** Dilute the Wash Buffer with 96-100% ethanol to a ratio of 1:4 (i.e. add 12 ml of Wash Buffer into 48 ml of 96-100% ethanol to form 48 ml of Working Wash Buffer solution).

Sample Preparation

This kit is suitable for use with EDTA, sodium citrate and heparin-anticoagulated fresh or frozen blood. For blood containing non-nucleated erythrocytes, it is recommended to use 5-250 µl as the sample volume, otherwise it is recommended to use 5-20 µl.

Assay Procedure

1. To a microcentrifuge tube, add blood (see Sample Preparation above for the recommended sample volume), 20 µl of Proteinase K and 500 µl of Binding Buffer. Mix for 15 seconds by vortexing.
2. If RNA-free genomic DNA is required, add 20 µl of RNase A, otherwise skip this step.
3. Allow to stand at room temperature for 10 minutes.
4. Centrifuge briefly. Add all the lysate to a spin column, then centrifuge at 12,000 × g for 1 minute. Discard the flow through.
5. Add 500 µl of Working Clean Buffer solution (with ethanol). Centrifuge at 12,000 × g for 30 seconds. Discard the flow through.
6. Add 500 µl of Working Wash Buffer solution (with ethanol). Centrifuge at 12,000 × g for 30 seconds. Discard the flow through. Repeat this step once more for a total of two times.
7. Place the spin column into a collection tube. Centrifuge the empty column at 12,000 × g for 2 minutes to remove any residual Working Wash Buffer solution. Air-dry the spin column at room temperature for several minutes.
8. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (preheated to 60 °C to increase DNA yield) or distilled water (pH > 7.0) to the center of the column. Allow to stand at room temperature for 1 minute.
9. Centrifuge at 12,000 × g for 1 minute to elute the genomic DNA. To recover more DNA, add Elution Buffer or distilled water again to perform a second elution. The second elution can be performed using the same microcentrifuge tubes or new sterile tubes. The purified DNA can be stored at -20 °C for long-term storage.

Notes

- All centrifugation steps should be carried out at room temperature.
- It is recommended to use fresh samples where possible, and to avoid repeated freeze/thaw cycles.
- Do not overload the column, as this may lead to a significantly lower yield than expected.
- Use sterile tubes and pipette tips to avoid DNase contamination.