For general laboratory use

Gene Transfer Pioneers
(GTP)

Blood DNA Extraction Kit

Kit for 50 purifications

Catalogue Number: DB10050

Store the kit at +15 to +25°C.

Introduction

The **Blood DNA Extraction Kit** provides an efficient method for purifying total DNA (including genomic, mitochondrial and viral DNA) from whole fresh blood. Chaotropic salt is used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed within 25 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA, with approximately 20-30 kb, is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the **Blood DNA Extraction Kit** is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of whole fresh human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

All solutions are clear. If any solution contains a precipitate, do not use it. Instead, warm the solution at room temperature or in a 37°C water bath to dissolve the precipitate.

Vial	Label	Contents
1. White Cap	RBC Lysis Buffer	60 ml
2. White microtube	RNase A	110 µl
3. Red Cap	DB Buffer	15 ml
4. Blue Cap	Wash Buffer	6 ml; add 24 ml ethanol
5. Black Cap	Elution Buffer	15 ml
6. Colorless Bag	DNA Spin Column	50 Columns

Storage and Stability

Store the **Blood DNA Extraction Kit** components at +15 to +25°C. Kit components are guaranteed to be stable date printed on the label.

Application

Isolation of up to 20 µg purified DNA from blood, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, sequencing, *in vitro* transcription or labeling reactions.

Before You Begin: Preparation of Working Solutions

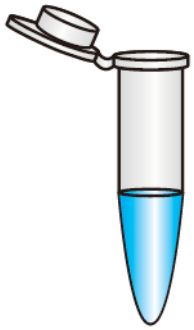
Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution:

Content	Preparation	Storage/ Stability
Wash Buffer (Blue Cap)	Add 24 ml absolute ethanol to Wash Buffer. * Label bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through expiration date printed on kit label.

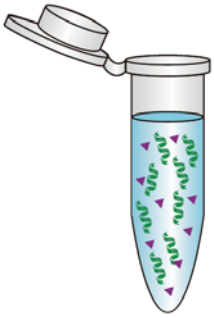
Caution

DB Buffer contains guanidine hydrochloride. During operation, always wear a lab coat, disposable gloves, and protective goggles.

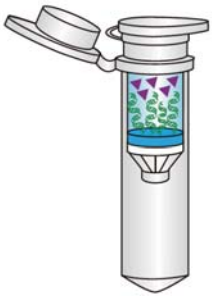
Quick Protocol Diagram



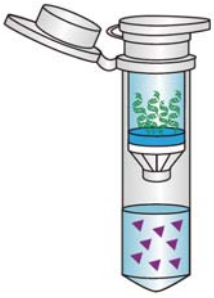
RBC lysis and WBC suspension



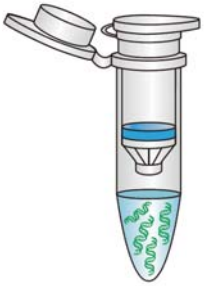
Lysis



DNA Binding



Wash



Elution

Blood DNA Extraction Kit Protocol

1. Transfer up to 300 µl of blood to a 1.5 ml microcentrifuge tube. Add 900 µl of RBC Lysis Buffer then mix by inversion. Do not vortex.

2. Incubate the tube for 5 minutes at room temperature. Centrifuge for 5 minutes at 3,000 x g then remove the supernatant completely.

3. Add 100 µl of **RBC Lysis Buffer** (**White Cap**) to resuspend the leukocyte pellet then proceed with Cell Lysis.

4. Add 200 µl of **DB Buffer** (**Red Cap**) to the 1.5 ml microcentrifuge tube then shake vigorously. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes.

5. Following 60ºC incubation, add 2 µl of **RNase A** (20 mg/ml) to the clear lysate then mix by shaking vigorously. Incubate at room temperature for 5 minutes.

6. Add 200 µl of **absolute ethanol** to the lysate then immediately mix by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette.

7. Transfer the mixture (including any precipitate) into a **DNA spin column** assembled in a clean collection tube (provided). Centrifuge at 10000 g for 2 min at room temperature.

8. Disconnect the **DNA spin column** from collection tube and discard the flow through solution. Reconnect the **DNA spin column** to the same collection tube.

9. Add 500 µl **Wash Buffer** (**Blue Cap**) to DNA spin column and centrifuge at 10000 g for 1 min. Discard flow through.

10. Centrifuge the **DNA spin column** at 10000 g for 1 min to remove residual ethanol.

11. Place the **DNA spin column** into the clean new microtube. Add 30 µl of pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

12. Centrifuge at 10000 g for 1 min.

13. Add another 30 µl pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

14. Centrifuge at 10000 g for 1 min.

15. Check 5-10 µl of extracted DNA by 1% agarose gel electrophoresis.

16. Store DNA at 4˚C or -20˚C.

Related DNA Extraction Products

Product	Package Size	Catalogue Number
Plasmid DNA Isolation Kit	25 preps	DV01025
Plasmid DNA Isolation Kit	50 preps	DV01050
Plasmid DNA Isolation Kit	100 preps	DV01100
DNA Gel Recovery Kit (PCR Purification Kit)	25 prep	DR02025
DNA Gel Recovery Kit (PCR Purification Kit)	50 preps	DR02050
Genomic DNA Extraction Kit from Gram Negative Bacteria	25 prep	DM04025
Genomic DNA Extraction Kit from Gram Negative Bacteria	50 prep	DM04050
Genomic DNA Extraction Kit from Gram Positive Bacteria	25 prep	DM05025
Genomic DNA Extraction Kit from Gram Positive Bacteria	50 prep	DM05050
Genomic Yeast DNA Extraction Kit	25 prep	DY06025
Genomic Yeast DNA Extraction Kit	50 prep	DY06050
Plant Total DNA Extraction Kit	50 prep	DP07050
Plant Total DNA Extraction Kit	100 prep	DP07100
Genomic DNA Extraction Kit from Seed	50 prep	DS08050
Genomic Fungi DNA Extraction Kit	25 prep	DF09025
Genomic Fungi DNA Extraction Kit	50 prep	DF09050
Blood DNA Extraction Kit	25 prep	DB10025
Blood DNA Extraction Kit	50 prep	DB10050
Blood DNA Extraction Kit	100 prep	DB10100
Tissue & Cells DNA Isolation Kit	50 prep	DT11050

www.irgtp.com
Tel: 021 – 88200114
021 – 88200112