SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318 E-mail: sales@sunlongbiotech.com
www.sunlongbiotech.com

Blood Genomic DNA Extraction Kit (Ethanol precipitation)

Cat number: EXD0211

Size: 50T/200T

Storage: Stored dry at room temperature (15-25°C) for up to 12 months. Stored at 2-8°C for longer storage.

Kit Contents

Component	50T	200T
Red Blood Cell Lysis Buffer,10×	30ml	120ml
Leukocyte Lysis Buffer	30ml	120ml
Protein Precipitate Buffer	30ml	120ml
TE buffer	15ml	60ml

Product Description

This kit is suitable for fresh sample or anticoagulanted blood, using isopropanol to precipitate the DNA. It is suitable for extraction of larger sample. The extracted DNA can be used in various routine operations, including restriction enzyme digestion, PCR, library construction, Southern hybridization and other experiments. For small sample we recommend to use D1800.

Reagent volumes required for processing different volumes of blood samples.

Note： According to dosage, dilute Red Blood Cell Lysis Buffer,10× to 1× before use.

Sample volume	1ml	5ml	10ml
Red Blood Cell Lysis Buffer, 1×	5ml	25ml	50ml
Leukocyte Lysis Buffer	0.5ml	2.5ml	5ml
Protein Precipitate Buffer	0.5ml	2.5ml	5ml
Isopropanol	1ml	5ml	10ml
75% Ethanol	1ml	5ml	10ml
TE buffer	100ul	0.5ml	1ml

Protocol

Isolation of genomic DNA from medium volume of whole blood(1-10 ml, take 1 ml as an example)

1. Sample preparation: Add 3ml (treble volume) Red Blood Cell Lysis Buffer, **1×**, (ensure that Red Blood Cell Lysis Buffer has been diluted) to 1ml whole blood sample, mix thoroughly by inverting up and down. Centrifuge for 1min at 12000rpm (For large extraction and large centrifuge, centrifuge at 11000rpm for 5min). Discard the supernatant. Add 2ml (double volume) Red Blood Cell Lysis Buffer, **1×**, mix well by pipetting. Centrifuge for 1min at 12000rpm.

Discard the supernatant. The precipitation is leukocyte pellet.

2. Add 500µl Leukocyte Lysis Buffer to the pellet, close the tube and vortex immediately until the pellet is completely homogenized. Incubate at 65°C for 10-20 min. In the meantime, invert for several times till the solution is clear.
3. Add 500µl Protein Precipitate Buffer, mix thoroughly by inverting until the DNA precipitate becomes visible. Incubate at 65°C for 5min. Centrifuge for 5min at 12000rpm. Transfer the supernatant to a new tube (avoid the precipitate or insoluble matter, if it occurred, re-centrifuge the solution)
4. Add 1ml Isopropanol, mix thoroughly by inverting until the DNA precipitate becomes visible. Centrifuge for 5min at 12000rpm. Discard the supernatant.
5. Add 1ml 75% Ethanol. Centrifuge for 5min at 12000rpm. Discard the supernatant
6. Allow the column to air dry with the cap open for several minutes to dry the membrane at room temperature or 50℃. Otherwise, ethanol will affect subsequent experiments such as enzyme digestion and PCR.

   Note: Avoid over-drying the DNA pellet, since over-dried DNA is very difficult to dissolve.
7. Add 100-300µl TE buffer, dissolve the DNA by incubating at room temperature.

   Note: If the DNA is not completely dissolved, incubate the solution overnight at room temperature (15-25°C) or at 50-60°C in water bath for 5min.

Note

1. Avoid repeated freezing and thawing of samples. Otherwise, the extracted DNA fragments are smaller and the extracted amount is also decreased.
2. If the precipitate occurs in the kit components, re-dissolved in 65℃ water bath before use, which will not affect the results.
3. Detect the concentration and purity of DNA: The purity of plasmid DNA influenced by many factors, the DNA purity can be detected by Agarose gel electrophoresis and Ultraviolet spectrophotometer. DNA should have absorption peak in OD_{260}, OD_{260}=1 is equal to 50µl double-stranded DNA, 40µl single-stranded DNA. OD_{260}/OD_{280} should be 1.7-1.9, the value will be lower if using distilled water in eluting, but do not show the purity is low.