Ver. 24111

ODP301

Genomic DNA Extraction Teaching Kit

For extraction of genomic DNA from blood and animal tissues

ISO 13485:2016 ISO 9001:2015

Genomic DNA Extraction Teaching Kit

Kit Contents

Contents	10 Preps
Buffer WLB	3mL
10X RBC Lysis Buffer	3mL
Buffer PB	2mL
Buffer TE	2mL
RNase A (10mg/mL)	20µL
6X DNA Loading Buffer	30µL
Agarose	4.8g
50X TAE	120mL
Collection Tubes, Polypropylene (1.5mL)	20 Nos.

Storage

Genomic DNA Extraction Teaching Kit could be stored dry at room temperature (15-25°C) for up to 15 months without showing any reduction in performance and quality. For long term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Introduction

Genomic DNA Extraction Teaching Kit is based on silica membrane technology and special buffer system for many kinds of sample's genomic DNA extraction. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Genomic DNA Extraction Teaching Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Materials Required but Not Provided:

Glass wares: Conical flask, Measuring cylinder.

Reagents: Distilled water, Ethidium bromide (10mg/mL), Ethanol, Isopropanol.

Other requirements: Electrophoresis apparatus, UV Transilluminator, Micropipettes, Vortex Mixer, Tips, Adhesive tape.

Yield of Genomic DNA Extraction Teaching Kit

Source	Yield
Whole blood from mammals (100-400µL)	2-10µg
Whole blood from bird or amphibian (5-20µL)	5-40µg
Tissue (25mg)	10-30µg

Important Notes

1. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
2. Increasing the time of absorption and elution could improve recovery efficiency.
3. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.

Protocol

1. Preparation of samples
   a) Blood: Take 300µL of fresh whole blood in a 1.5mL collection tube. Ensure that the blood sample is at room temperature (15-25˚C) before beginning the protocol. Add 900µL of 1X RBC lysis buffer and mix well by inverting the tubes for 6-8 times. Incubate at room temperature for 5 minutes. Mix the contents intermittently by inverting 2-3 times during incubation. Centrifuge the tube at 15000 rpm for 1 minute at room temperature. Discard the supernatant carefully without disturbing the white pellet such that very small amount of residue liquid remains back in the tube.
   Note: If some red blood cells or cell debris are observed along with the WBC pellet, resuspend the pellet in 600µL of 1X RBC lysis buffer. Incubate at room temperature for 2 minutes. Pellet down the WBC pellet by repeating above centrifugation step.
   b) Tissue: Take finely chopped 30mg tissue (preferably chicken liver or intestine).
2. Add 300µL of Buffer WLB to the WBC pellet.
   Note:
   - **For blood samples, gently pipette to lyse the cells. Solution should become viscous. If any cell clumps are still present, incubate the solution at 37˚C (10 minutes) or until the clumps dissolve.**
   - **For tissue samples, homogenize the tissue with micro pestle, then vortex the mixture.**
3. Add 1.5µL RNase A solution. Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for 10 minutes at 37˚C. Cool the sample to room temperature before further processing.
4. Add 200µL of Buffer PB to the lysate and mix by vortexing for 30 seconds. Incubate on ice for 5 minutes.
5. Centrifuge at 14000 rpm for 3 minutes at room temperature.
   Note: For blood sample, protein will precipitate and form a compact dark brown pellet. If the pellet is not compact, then incubate on ice for 5 minutes and repeat above centrifugation step as mentioned.
6. Transfer the above supernatant to a new 1.5mL micro-centrifuge tube. Add 300µL of 100% isopropanol and mix by inverting the tube gently for 2 minutes.

7. Centrifuge at 15000 rpm for 1 minute at room temperature. Small white pellet of DNA will be visible. Discard the supernatant without disturbing the pellet.
8. Add 300µL 70% ethanol to the DNA pellet and wash by inverting the tube 6-7 times.
9. Centrifuge at 15000 rpm for 2 minutes at room temperature. Carefully discard the supernatant without disturbing the pellet. Repeat the wash step one more time.
10. Invert the tube on a clean tissue paper and air dry the pellet for 10-15 minutes.
11. Add 100µL Buffer TE and vortex for one minute. Incubate the tube at 65˚C for 10 minutes to dissolve the DNA pellet completely.

Note: For long-term storage of DNA, storing at –20°C is recommended.

Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500mL of 1X TAE buffer, add 10mL of 50X TAE Buffer to 490mL of sterile distilled water. Mix well before use.

Preparation of agarose gel: To prepare 0.8% agarose gel, add 0.4g agarose in 50mL of 1X TAE buffer in a glass beaker or flask. Heat the mixture in a microwave or hot plate or burner by swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool down to about 55-60°C. Add 0.5µL Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

Note: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

Loading of the DNA samples: To prepare sample for electrophoresis, add 2µL of 6X DNA Loading Buffer to 10µL of DNA sample. Mix well by pipetting and load the sample into the well. Load the Control DNA after extracting the DNA sample.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

Quantification of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260nm, 280nm, and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260nm to absorbance at 280nm. The concentration of DNA is calculated by the following formula:

Concentration of DNA sample (µg/mL) = $100 \times A_{260} \times$ dilution factor