Ver. 24101

ODP301

Genomic DNA Extraction Teaching Kit

For extraction of genomic DNA from blood, cells and animal tissues

ISO 13485:2016 ISO 9001:2015

Genomic DNA Extraction Teaching Kit

Kit Contents

Contents	10 Preps
Buffer GA	2mL
10X RBC Lysis Buffer	2mL
Buffer GB	2mL
Isopropanol	4mL
Buffer TE	2mL
Proteinase K (20mg/mL)	200µL
Agarose	4.8g
50X TAE	120mL
Collection Tubes, Polypropylene (2mL)	30N

Storage

Genomic DNA Extraction Teaching Kit could be stored dry at room temperature (15-25°C) for up to 12 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)

Proteinase K should be stored at -20°C from receipt of the kit.

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

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) Non-nucleated: Pipette 200µL sample to a microcentrifuge tube. If the volume is less than 200µL, adjust volume to 200µL with Buffer GA. If the sample volume is more than 200µL, e.g., 300µL-1mL, add 3 times volume Red Cell Lysis Buffer (Cat. No. ORT122, Not supplied) to the sample, close the cap and invert the tube. Place the tube in room temperature (15–25°C) for5 minutes, and centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, then discard the supernatant and pipette 200µL Buffer GA and mix by pulse-vortexing.
   Note: If some red blood cells or cell debris are observed along with the white blood cell pellet, resuspend the white blood cell pellet in 600µL of 1X RBC Lysis Buffer. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm (~13,400 × g) for 1 minute to pellet down the white blood cells.
   b) Nucleated: Add 5-20µL anticoagulated blood; adjust volume to 200µL with Buffer GA.
   c) Tissue: Cut up to 25mg tissue (up to 10mg spleen) into small pieces and place in a 1.5mL microcentrifuge and add 200µL Buffer GA. If required samples can be homogenized in liquid nitrogen or by using grinding pestle (Cat. no. OWL046, Not supplied) before addition of Buffer GA
2. Add 20µL Proteinase K, mix thoroughly by vortexing. Incubate at 56°C until the tissue is completely lysed.
   Note: For blood samples, ideal incubation time is 10 minutes. Lysis time varies depending on the type of tissue processed.
3. Add 200µL Buffer GB to the sample, mix thoroughly by vortexing. Briefly centrifuge the 1.5mL microcentrifuge tube at 1,000 - 3,000 rpm for 30 seconds to remove drops from the inside of the lid.
   Optional: Incubate at 70°C for 10 minutes to yield a homogeneous solution.
   Note: Precipitates are expected, but it will not interfere with the extraction.
4. Add 300µL of 100% isopropanol and mix by inverting the tube gently till the DNA in white fibrous form is visible (30-40 times).

5. Centrifuge at 12,000 rpm for 1 minute at room temperature. Small white pellet of DNA will be visible. Discard the supernatant.
6. Add 200µL ethanol (96-100%) to the sample, and mix by vortexing for 20 seconds. Centrifuge at 12,000 rpm for 2 minutes at room temperature. Carefully discard the supernatant.
   Note:The pellet may be very loose at this point, so the supernatant should be carefully discarded without disturbing the pellet. Repeat the step for one more time.
7. Pipette 50–200µL Buffer TE directly onto the membrane, incubate for 2-5 minutes at room temperature (15–25°C), and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to elute.
   Note: To increase the DNA yield,
   - **Introduce the eluted Buffer TE to the tube and Centrifuge for 2 minutes at 12,000 rpm.**
   - **Warm the Buffer TE at 50-60°C before adding to the tube. If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting; we suggest Buffer TE or distilled water (pH 7.0 - 8.5) to elute genomic DNA. For short-term storage of the DNA, 2-8°C and for long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.**
   - **Sometimes fragments are seen after 10 minutes of incubation. In this case, increase the incubation time till the fragments dissolve completely. Some samples may need incubation for 1-2 hours at 65°C to rehydrate the DNA.**
   - **Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.**

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 gel 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 x A_{260} x dilution factor