Ver. 260201

ODP301

Genomic DNA Kit

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

Origin

ISO 13485:2016 ISO 9001:2015

Genomic DNA Kit

Spin Column

Kit Contents

Contents	50 Preps
Buffer GA	10mL
Buffer GB	10mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Spin column CB3	50
Collection Tubes (2mL)	50

Storage

Genomic DNA Kit could be stored dry at room temperature (15-25°C) 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 Kit is based on silica membrane technology and special buffer system for many kinds of sample's genomic DNA extraction. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. 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 Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Yield of Genomic DNA

Source	Yield
Whole blood from mammals (100-400µL)	2-10µg
Whole blood from bird or amphibian (5-20µL)	5-40µg
Culture cells (10^6 – 10^7 cells)	5-30µg
Tissue (25mg)	10-30µg

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
2. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.
5. If a precipitate has formed in Buffer GB or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

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) for 5 minutes, and centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, then discard the supernatant and pipette 180µL Buffer GA and mix by pulse-vortexing.
   b) Nucleated: Add 5-20µL anticoagulated blood; adjust volume to 200µL with Buffer GA.
   c) Cultured cells: Centrifuge the cell for 1 minute at 12,000 rpm (~13,400 ×g), then discard the supernatant and resuspend cell pellet in 200µL Buffer GA.
   d) Tissue: Cut up to 25mg tissue (up to 10mg spleen) into small pieces and place in a 1.5mL microcentrifuge and add 180µL 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 200µL ethanol (96-100%) to the sample, and mix by vortexing for 20 seconds. 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.
   Note: Precipitate formed in the earlier step will be dissolved.
5. Pipette the mixture from step 4 into the spin column CB3 (in a 2mL collection tube) and incubate at room temperature for 5-10 minutes. Centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds and discard flow-through. Place the spin column CB3 into the collection tube
   Note: Since the capacity of spin column CB3 is 700µL, the centrifugation step must be repeated for processing all the mixture from step 4.
6. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
7. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
8. Repeat step 7.
9. Centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to remove residual wash Buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5mL or 2mL microcentrifuge tube. Open the lid of the spin column CB3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.
   Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
10. 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 spin column CB3 and centrifuge for 2 minutes at 12,000 rpm**.
    - **Warm the Buffer TE at 50-60°C before adding to the spin column CB3.**

    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 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.