PURO-Genomic DNA

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

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PURO-Genomic DNA

Cat. no. SC04

Kit Contents

Contents	SC04-01 50 preps	SC04-02 200 preps
Buffer GA	15 ml	50 ml
Buffer GB	15 ml	50 ml
Buffer GD	13 ml	52 ml
Buffer PW	15 ml	50 ml
Buffer TE	15 ml	60 ml
Proteinase K	1 ml	4×1 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Compatible Reagents

Red Cell Lysis Buffer; RNaseA (100 mg/ml)

Storage

PURO Genomic DNA can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.

Introduction

PURO Genomic DNA is based on silica membrane technology and special buffer system for many kinds of sample's gDNA 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. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA purified by PURO Genomic DNA is highly suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Yield of Genomic DNA with TIANamp Genomic DNA Kit

Source	DNA Yeild
Whole blood from mammalian (100 µl-400 µl)	3-10 µg
Whole blood from bird or amphibian (5-20 µl)	5-40 µg
Cultured cells (10^6-10^7 cells)	5-30 µg
Tissue (30 mg)	10-30 µg

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
2. If a precipitate has formed in Buffer GA or Buffer GB, warm buffer to 56°C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-25°C).

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 (For blood with nonnucleated erythrocytes, follow step a; for blood with nucleated erythrocytes, follow step b; for cultured cells, follow step c; for tissue, follow step d.)

a. Nonnucleated: Pipet 200 µl sample to the microcentrifuge tube. If the volume is less than 200 µl, adjust volume to 200 µl with buffer GA. If the sample volume is more than 200 µl, e.g. 300 µl-1 ml, please refer the following step: add 3 times volume Red Cell Lysis Buffer to the sample, then invert the tube and close the cap. Stay the tube in room temperature (15–25°C) for 5 min, and centrifuge at 12,000 rpm (~13,400 × g) for 1 min, then discard the flow-through and pipet 200 µl buffer GA and mix by pulse-vortex.

b. Nucleated: Add 5-20 µl anticoagulated blood; adjust volume to 200 µl with buffer GA.

c. Cultured cells: Centrifuge the cell for 1 min at 12,000 (~13,400 × g), then discard the flow-through and re-suspend cell pellet in 200 µl buffer GA.

d. Tissue: Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces and place in a 1.5 ml microcentrifuge, centrifuge at 12,000 rpm (~13,400 × g) for 1 min , then discard the flow-through and re-suspend cell pellet in 200 µl buffer GA.

Optional: RNase treatment of the sample. Add 4 µl of RNase A (100 mg/ml), mix by vortex, and incubate for 5 min at room

temperature (15-25°C).

2. Add 20 µl Proteinase K, mix thoroughly by vortex.
   If the sample is tissue: incubate at 56°C until the tissue is completely lysed.
   Note: Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1-3 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.
3. Add 200 µl Buffer GB to the sample, mix thoroughly by vortex, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
4. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
5. Pipet the mixture from step 4 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.
6. Add 500 µl Buffer GD to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
7. Add 700 µl Buffer PW to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
8. Add 500 µl Buffer PW to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.

9. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.

   Note: The resident ethanol of buffer PW may have some affection in downstream application.

10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

    Note: If the volume of eluted buffer is less than 50 µl, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest chose buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. 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.