PURO
Genomic DNA

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

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PURO-Genomic DNA

Cat. no. SA05

Kit Contents

Contents	SA05-01 50 preps	SA05-02 100 preps
Buffer GL	10 ml	20 ml
Buffer GA	10 ml	20 ml
Buffer GB	10 ml (+ 40 ml of EtOH 96%)	20 ml (+ 80 ml of EtOH 96%)
Buffer GC	10 ml	20 ml
Proteinase K	1 ml	2 × 1 ml
Spin Column	50	100
Collection Tubes 2 ml	50	100
Handbook	1	1

Additional equipment and reagents required

Phosphate buffered saline (PBS)
Microcentrifuge
RNAse A (10 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 PURO Genomic DNA Kit

Source	DNA Yield
Whole blood from mammalian (100 µl - 400 µl)	3 - 10 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, 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 GB has been prepared with appropriate volume of ethanol (96 - 100%) as indicated on the bottle and shake thoroughly.

1. a) Add 200 µl of **whole blood** to the microcentrifuge tube. If the sample volume is less than 200 µl, add the appropriate volume of PBS.

b) For **cultured cells**, harvest 5 x 10^6 cells and centrifuge for 5 min at 500 xg in a 1.5 ml microcentrifuge tube. Aspirate the medium and resuspend cell pellet in 200 µl of PBS.

c) 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 resuspend tissue pellet in 180 µl buffer GL.

Add 20 µl **Proteinase K** (20 mg/ml), mix thoroughly by vortex and incubate at 56 °C until the tissue is completely lysed. Continue on step 3.

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

Optional: RNase treatment of the sample. Add 4 µl of RNAse A (10 mg/ml), mix by vortex and incubate for 5 min at room temperature (15 - 25 °C).

2. Add 20 µl **Proteinase K** (20 mg/ml), mix thoroughly by vortex.
3. Add 200 µl Buffer **GA** to the sample, mix thoroughly by vortex,

and incubate at 56 °C for 15 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

Note: Do not add Protease K directly to Buffer GA.

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 (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 GB (prepared with appropriate volume of ethanol 96 - 100%)** to Spin Column 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. Optional repeat step 6.
8. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 min to dry the membrane completely.

   Note: The resident ethanol of buffer GB may have some affection in downstream application.
9. Place the Spin Column in a new clean 1.5 ml microcentrifuge tube and pipet 50 - 200 µl **Buffer GC** 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, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest

choose buffer GC or distilled water (pH 7.0 - 8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer GC and storing at -20 °C is recommended, since DNA stored in water is subject to acid hydrolysis.