

EXOBX® Genomic DNA Isolation Kit (Magnetic Bead-Based)

Cat. no. R6312

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Catalog Number	Reagent In Kit	Size	Storage
R6312	Lysis Buffer	30 mL	RT
	Magnetic Bead	2.5 ml	4 °C
	Elution Buffer D	10 mL	RT
	RNase A(for gDNA isolation)	1 tube	-20 °C
	Manual	1	

1. Transportation and Storage

- 1) This product is transported at low temperature by using ice bag, and the reagents in the kit were stored at different temperature according to the previous table.
- 2) RNase A is stored at -20 °C for long time, but at 4 °C for 6 months.
- 3) The other reagents in the kit are stored at room temperature, and valid for 2 years.

2. Important Product Information

- 1) The magnetic beads should be stored at 4 °C and are forbidden to freeze, or they become invalid.
- 2) The volume of elution buffer (or ddH₂O) should not be less than 100 µl, otherwise the yield of genomic DNA will be reduced.
- 3) The normal range of OD₂₆₀/OD₂₈₀ for genomic DNA (gDNA) is 1.7-1.9 (eluted with ddH₂O) or 1.8-2.0 (eluted with Elution Buffer D). When the ratio is greater than 2.0, the isolated gDNA may contain RNA. However, if the ratio is less than 1.7, the isolated gDNA may be contaminated by protein.
- 4) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

The EXOBX®™ Genomic DNA Isolation Kit enables rapid isolation of genomic DNA from cultured mammalian cells and tissues without using proteinase K digestion. The isolated genomic DNA can be used for various subsequent experiments, including enzyme digestion, PCR, sequencing and other experiments.

4. Additional Materials Required

- 75% ethanol
- Variable-speed bench-top microcentrifuge (refrigerated)
- Handheld Homogenizer
- Vortex Mixer
- Magnetic Racker

- Rotary Mixer

5. Experimental Procedure

- 1) Sample preparation.
 - a) Cell sample. Pellet $1\sim5 \times 10^6$ cells by centrifuging harvested cell suspension in a 1.5 mL microcentrifuge tube at 1000 g for 3 minutes. Carefully remove and discard the supernatant. Add 0.5 ml Lysis Buffer and 5 μ L RNase A, mix thoroughly and incubate at room temperature for 5 minutes.
 - b) Tissue sample. Tissues (10-20 mg) were cut into pieces, suspended with 0.5 ml Lysis Buffer and 5 μ L RNase A, and then homogenized until the mixture is transparent by using Handheld Homogenizer. Incubate at room temperature for 5 minutes.
- 2) Add 50 μ L magnetic beads (vortex the magnetic beads before use) to the lysates, mix and incubate for 5-10 minutes at room temperature by using a rotary mixer.
- 3) Pellet magnetic beads by centrifugation at 3000 g for 5 min, or by using the magnetic rack. Carefully remove and discard the supernatant.
- 4) Add 500 μ L of 75% ethanol, and vortex for 5-10 sec to completely disperse the magnetic beads.
- 5) Pellet the magnetic beads by centrifugation or using the magnetic rack. Carefully remove and discard the supernatant.
- 6) Repeat the washing steps (step 4 and 5) to wash the magnetic beads again.
- 7) Centrifuge at 3000 g for 1 min, and remove the residual liquid with a pipette.
- 8) Add 100 μ L Elution Buffer D (or ddH₂O), and vortex for 30 sec.
- 9) Centrifuge at 7000 g for 2 min, and then put the centrifuge tube on a magnetic rack, and then transfer the supernatants to new tubes, which is the genomic DNA solutions.
- 10) The isolated genomic DNA can be immediately used for subsequent experiments or stored at -20 °C.

6. Identification of Isolated genomic DNA

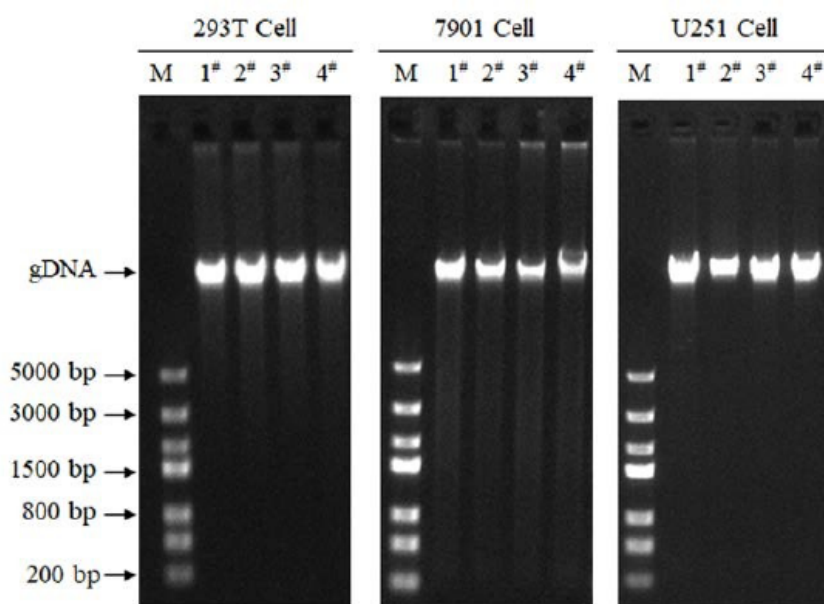


Figure 1. Identification of isolated genomic DNA by agarose gel electrophoresis.

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The concentration of isolated genomic DNA is low.	Less samples were used to isolate genomic DNA.	Increase the samples during genomic DNA isolation.
	Samples have not been disrupted by Lysis Buffer completely, and the genomic DNA in cells and tissues has not been released fully.	Add enough Lysis Buffer to the samples, mix or homogenize thoroughly, and incubate at room temperature for long time.
	Reagent becomes invalid.	Use the reagents within the shelf life.
	Magnetic beads have been frozen and become invalid	Use new magnetic beads.
	The pH value of eluent is not suitable for eluting genomic DNA from magnetic beads.	Please use Elution Buffer D, or ddH ₂ O with pH values between 7.0-8.0.
	The incubation time between samples and magnetic beads is short.	Prolonged incubation time.
	Elution intensity is low.	Increase vortex intensity and prolong vortex time.
The isolated genomic DNA has RNA contamination.	Eluent volume is too small.	Increase the volume of Elution Buffer D or ddH ₂ O.
	The RNase A becomes invalid.	Use new RNase A.