

MagExtractor -*Genome*-

NPK-101 100 preparations
Store at 4°C

Contents

[1]	Introduction
[2]	Components
[3]	Materials required
[4]	Protocol
	1. Purification from whole blood or cultured cells
	2. Purification from animal tissue or mouse tail
[5]	Troubleshooting
[6]	References
[7]	Related products

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI BIOTECH, CO., LTD.)
Tel(86)-21-58794900

[1] Introduction

Description

MagExtractor -*Genome*- provides a simple and reliable method for the rapid purification of genomic DNA from various specimens (e.g. whole blood, cultured cells or animal tissues etc) using magnetic silica beads. This kit is based on binding properties of DNA to a silica surface in the presence of chaotropic agents ¹⁾²⁾. The purified genomic DNA can be used directly for PCR experiments.

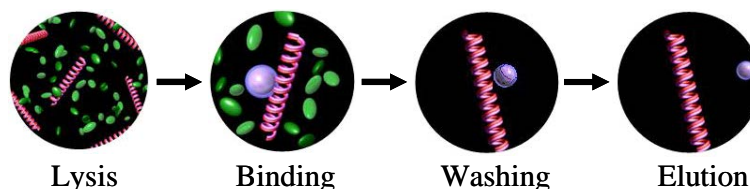


Fig. 1 Principle of purification

Features

- This kit can be applied to extraction of DNA from whole blood specimens. A leukocyte or lymphocyte separation step is not necessary.
- This kit does not contain hazardous substances, such as phenol or chloroform.
- Purified genomic DNA can be eluted in sterilized water. Therefore, the purified DNA samples can be applied directly to other methods, such as PCR, etc.
- This kit is suitable for high-throughput extraction of genomic DNA from various specimens. The following table shows the typical yield and purity in each case.

Sample	Yield	Purity (A260/280)	Application
Blood	2 µg/100µL whole blood	1.8 ± 0.1	PCR
Cultured cells	3 µg/5x10 ⁵ HeLa cells		
Tissue	5 µg/5 mg tissue (swine testis)		
Mouse tail	3 µg/2 mm tail		

Notes

In the case of tissues or mouse tails, homogenization and/or lysis steps are necessary prior to extraction.

[2] Components

This kit contains the following components for 100 preparations. All reagents should be stored at 4 °C.

Lysis & Binding Solution	100 mL
Washing Solution	200 mL
Magnetic Beads	5 mL

Caution:

- The “Lysis & Binding Solution” and “Washing Solution” contain chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. If contact with skin occurs, wash thoroughly with water. If the eyes get affected, flush thoroughly for 15 min. with cool water, and consult a physician.

Notes:

- All reagents should be used at room temperature for extraction.
- If used within one month or less, all components can be stored at room temperature (≤25°C).

[3] Materials required

The following materials are required for purification.

- (1) Reagents
 - Sterilized water
 - 70% Ethanol
- (2) Instruments
 - Tube mixer
 - Magnetic stand

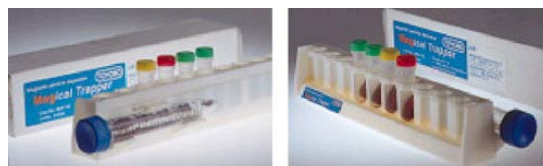


Fig.2
Magnetic stand
 Magical Trapper (Code No.MGS-101)

[4] Protocol

1. Purification from whole blood or cultured cells
<Standard protocol>

(1) Preparation of specimens

-Whole blood

Dispense 100 μ L of whole blood into 1.5-mL microtubes.
 (Whole blood can be used directly.)

-Cultured cells

Collect cultured cells by centrifugation in PBS(-), and dispense 1×10^5 - 10^6 cells/100 μ L PBS(-) into 1.5-mL microtubes.

(2) Add 750 μ L **Lysis & Binding Solution**.

(3) **[Binding]** Add 40 μ L **Magnetic Beads** and mix well for 10 minutes using a tube mixer.

Note

Suspend **Magnetic Beads** completely prior to use.

(4) Place each tube into the magnetic stand. The magnet will attract the magnetic beads, separating from the specimen solution.



Fig. 3
Magnetic separation

(5) After magnetic capture, carefully remove the supernatant.

(6) **[Washing]** Add 900 μ L **Washing Solution** to the beads and vortex for 5 seconds (Maximum speed).

(7) Place the tube on a magnetic stand and collect the beads for 30 seconds.

(8) After magnetic capture, carefully remove the supernatant.

(9) **[Washing]** Repeat (6) - (8)

(10) **[Washing]** Add 900 μ L 70% Ethanol and vortex 5 seconds (Maximum speed).

- (11) Place the tube on a magnetic stand and collect the beads for 30 seconds with the magnet.
- (12) After magnetic capture, carefully remove the supernatant.
- (13) **[Washing]** Repeat (10) - (12)
- (14) **[Elution]** Add 100 μL sterilized water and mix well for 10 minutes using a tube mixer.
- (15) Place the tube in the magnetic stand.
- (16) Collect the supernatant and place in a fresh tube.

2. Purification from animal tissue or mouse tails

- (1) Pretreatment of specimens

Animal tissue or mouse tails should be treated with either of the following methods.

(A) Homogenization of specimens in Lysis & Binding Solution.

Tissue ($\leq 10\text{mg}$) or mouse tail (2-5 mm)
 ↓
 ↓ ← 850 μL **Lysis & Binding Solution**
 ↓
 Homogenization
 ↓
 Centrifuge at 10,000 rpm for 5 minutes
 ↓
Supernatant 850 μL (Pretreated solution)

(B) Homogenization after Proteinase K treatment

Tissue ($\leq 10\text{mg}$) or mouse tail (2-5 mm)
 ↓
 ↓ ← 90 μL Proteinase K buffer [100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 25mM EDTA]
 ↓ ← 5 μL 10 mg/mL Proteinase K (30 U/mg)
 ↓ ← 5 μL 10% SDS
 ↓
 Incubate at 55°C for 6-18 hr. (Mix 2-3 times halfway through incubation)
 ↓
 Centrifuge at 10,000 rpm for 5 minutes
 ↓
 Supernatant 100 μL
 ↓ ← 750 μL **Lysis & Binding Solution**

Pretreated solution 850 μL

- (2) Perform the purification steps (3)-(16) in **Standard protocol**.

[5] Troubleshooting

Symptom	Cause	Solution
Low yield	Excess specimen	Excess samples decrease DNA yields. Use appropriate amount of specimen.
	Insufficient homogenization	-Insufficient homogenization of tissue or mouse tails decreases DNA yields. Prolong the homogenization step. -In the case of formalin-fixed tissue, Proteinase K pretreatments might be effective.
		-In the case of gram-negative bacteria or yeast, pretreatments using specific enzymes (e.g., zymolyase for yeast) are necessary. Gram-negative bacteria (e.g., <i>E. coli</i>) can be used directly.
Poor amplification on PCR	Ethanol inhibition	Decrease the amount of DNA solution for PCR by up to 20% of reaction volume. Purified DNA samples contain residual amounts of ethanol from the washing steps. The ethanol can be evaporated by heating sample at 75°C for 5 minutes.

[6] References

- 1) B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. USA*. 76: 615-619 (1979)
- 2) R. Boom, C. J. A. Sol, M. M. M. Salimans, C. L. Wertheim-van Dillen, P. M. E. Dillen and J. van der Noordaa, *J. Clin. Microbiol.*, 28: 495-503 (1990)

[7] Related products

Product name	Package	Code No.
Magnetic stand Magical Trapper	1 piece	MGS-101