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Instruction manual MagExtractor-Genome-0810 F0981K

MagExtractor -*Genome*-

NPK-101 100 preparations
Store at 4°C

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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.

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[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.

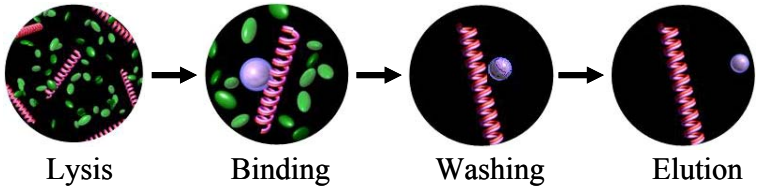

Fig. 1 Principle of purification

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/100 µl 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 extractionn.

[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).

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[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 µl 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 $1x10^5$-10^6 cells/100 µl PBS(-) into 1.5-ml microtubes.

(2) Add 750 µl **Lysis & Binding Solution**.

(3) **[Binding]** Add 40 µl **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.

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

Fig. 3
Magnetic separation

(6) **[Washing]** Add 900 µl **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 µl 70% Ethanol and vortex 5 seconds (Maximum speed).

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(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 (≤ 10mg) 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 (≤ 10mg) 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.**

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[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., *E. coli*) 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 **Mag*ical Trapper***	1 piece	MGS-101

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