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Instruction manual MagExtractor-RNA-2004

F0982K

MagExtractor -*RNA*-

NPK-201F 100 preparations
Store at 4°C

Contents

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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FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

MagExtractor -*RNA*- provides a simple and reliable method for the rapid purification of total RNA from various specimens (e.g. cultured cells or animal tissues) using magnetic silica beads. This kit is based on binding properties of RNA onto a silica surface in the presence of chaotropic agents[1)2)] and an RNA-binding accelerator. Purified total RNA contains primarily rRNA and mRNA. The purified total RNA can be used for RT-PCR experiments.

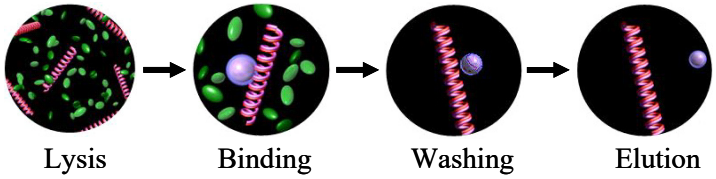

Fig. 1 Principle of purification

Features

-Purified total RNA contains primarily rRNA and mRNA.
-This kit does not contain hazardous substances, such as phenol or chloroform.
-No ethanol is used in the washing steps.
-This kit is suitable for high-throughput extraction of total RNA from various specimens. The following table shows the typical yield and purity in each case.

Sample	Amount of specimens	Yield	Remarks
Cultured cells	~5x10^6 cells	~10 µg/10^6 cells	Total RNA yields depend on the number of cells.
Tissue	~30 mg	~15 µg/30mg	Total RNA yields depend on tissues or storage conditions.
Yeast	~5 O.D. (660nm)/ mL	~20 µg/5 O.D.	Pretreatment by zymolyase is necessary.

Notes

-This kit is not applicable for extraction from whole blood or serum. In the case of whole blood, white blood cells should be separated by a centrifugation using Ficoll.

[2] Components

This kit contains the following components for 100 preparations. Lysis & Binding Solution and 2-mercaptethanol should be stored at 4 °C. The remaining components should be stored at 4°C or room temperature.

Lysis & Binding Solution*	77 mL
Washing Solution I	66 mL
Washing Solution II	176 mL
Elution Solution	10 mL
Magnetic Beads	6 mL

* Lysis & Binding Solution and 2-mercaptethanol (2-ME) should be mixed at a ratio of 100:1 prior to use. **2-ME is not supplied with this kit.**

Caution:

-The “Lysis & Binding Solution” and “Washing Solution I” 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.

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[3] Materials required

The following materials are required.

-2-Mercaptoethanol (2-ME)
- Magnetic stand
- Heating block can be set to 65°C.

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

[4] Protocol

1. Preparation of reagents required

(1) Lysis & Binding solution (2-ME)

Lysis & Binding Solution and 2-Mercaptethanol (2-ME) should be mixed at a ratio of 100:1 prior to use. The mixed solution can be stored at 4 °C for 3 months. **2-ME is not supplied with this kit.**

(2) Washing Solution I and II should be used at room temperature.

2. Pretreatment of specimens

Cultured cells, tissue, or yeast specimens should be treated with either of the following methods.

(1) Cultured cells

Pellet cultured cells by centrifugation in PBS(-). After resuspension with a small amount of PBS(-), dispense 1x10^5-10^6 cells/100 μL PBS(-) into 1.5-mL microtubes.

Cultured cells (≤ 5x10^6 cells)
↓
↓← 700 µL **Lysis & Binding Buffer** (containing 2-ME)
↓
Pipette until viscosity is decreased
↓
Vortex for 30 seconds
↓
Incubate at room temperature for 10-15 minutes

(2) Tissue

Freezing tissue (≤ 30mg)
↓
↓← 750-900 µL **Lysis & Binding Buffer** (containing 2-ME)
↓
Homogenization on ice
↓
Vortex for 30-60 seconds until viscosity is decreased
↓
Centrifuge at 3,000-5,000 rpm for 10 seconds
↓
Supernatant 700 µL

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(3) Yeast cells

Pellet of yeast cells from 1 mL culture medium (≤ 5 O.D. [660 nm] /mL)
↓
↓← 50 µL 30 mg/mL Zymolyase (20,000 U/g)*
↓
Incubate at 37°C for 5-20 minutes after vortexing
↓
↓← 700 µL **Lysis & Binding Buffer** (containing 2-ME)
↓
Pipette until viscosity is decreased
↓
Vortex for 30 seconds
↓
Incubate at room temperature for 10-15 minutes

*Zymolyase buffer : 0.9M Sorbitol, 0.1 M EDTA, 50 mM DTT (pH 7.5)

3. Purification

(1) **[Binding]** Add 50 µL magnetic beads to the 700 µL pretreated solution (see above section) and vortex for 20 seconds (maximum speed) and incubate for 40-60 seconds at room temperature.

Notes
Completely resuspend the magnetic beads prior to use.

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

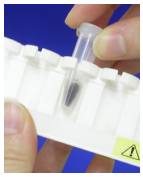

Fig. 3
Magnetic separation

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

(4) **[Washing]** Add 600 µL **Washing Solution I** to the beads and vortex for 10 seconds by maximum speed.

(5) Place each tube in the magnetic stand and collect the beads.

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

(7) **[Washing]** Add 800 µL **Washing Solution II** and vortex for 5 seconds by maximum speed.

(8) Place each tube in the magnetic stand and collect the beads.

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

(10) **[Washing]** Repeat (7) - (9)

(11) **[Elution]** Add 40 µL **Elution Solution** and mix well.

(12) Heat at 65°C for 2 minutes, and mix well.

(13) Place the tube in the magnetic stand.

(14) Collect the supernatant into a fresh tube.

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[5] Related Protocol

1. DNase I treatment of total RNA

Total RNA prepared by general methods contains genomic DNA. Genomic DNA can be eliminated by the following method.

(1) Mix the following reagents.

Nuclease-free water	X µL
Total RNA (<10 µg)	Y µL
10 x DNase I Buffer [*e.g.* 100 mM Tris-Cl, 20 mM $MgCl_2$(pH 7.5)]	1 µL
RNase-free DNase I (10 U/µL)	0.5 µL
Total volume	10 µL

(2) Incubate on ice for 10-30 min.

(3) Purify the treated RNA according to the following step.

DNase I-treated RNA
↓ ← Add nuclease-free water (adjust volume to 100 µL)
↓ ← Add 100 µL TE-saturated phenol

Vortex
Keep on ice for 5 min.
↓ Centrifuge at 12,000 rpm for 5 min.

Supernatant
↓ ← Add 100 µL chloroform: isoamyl alcohol (24:1), Vortex
↓ Centrifuge at 12,000 rpm for 5 min. at 4 °C

Supernatant
↓ ←Add 100 µL 5 M ammonium acetate + 200 µL isopropanol
+ [5 µL 2 mg/mL glycogen* (for coprecipitation) : optional]
Vortex
Incubate at - 20 °C for 30 min.
↓ Centrifuge at 12,000 rpm for 10-15 min. at 4 °C

Discard supernatant
Precipitate
↓ ←Add 1 mL 70% ethanol
↓ Centrifuge at 12,000 rpm for 5 min.

Discard supernatant
Precipitate
↓ ←Dissolve in appropriate volume of nuclease-free water

RNA solution

*Molecular biology grade

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[5] Troubleshooting

Symptom	Cause	Solution
Low yield	Insufficient pipetting in Lysis & Binding Solution	Pipette specimens in Lysis & Binding Solution until viscosity is decreased.
	Insufficient incubation in Lysis & Binding Solution	Increase incubation time > 15 minutes.
	Excess centrifugation of treated solution in Lysis & Binding Solution	In order to remove insoluble tissue, centrifuge at ≤ 5,000 rpm for 10 seconds.
Low 260/280 nm ratio	Excess specimen	Decrease the amount of specimen.
	Low RNA concentration	Low RNA concentration tends to result in low 260/280 nm ratio. Dilute the RNA with 10 mM Tris-HCl (pH 8.0)
Poor amplification with RT-PCR	Contamination with genomic DNA	Treat the RNA with DNase I. (see [5])
	RNA degradation	-Confirm storage condition of specimens for purification. -See “Degradation of RNA”
Degradation of RNA	Heating of RNA	Heating of RNA in DNase I buffer may accelerate RNA degradation.
	Excess specimen	RNA extracted from excess specimens might contain residual ribonuclease.

[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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