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Instruction manual MagExtractor-Plasmid-2004 F0983K

MagExtractor -*Plasmid*-

NPK-301 500 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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[1] Introduction

Description

MagExtractor -Plasmid- provides a simple and reliable method for the rapid purification of plasmid DNA from *E. coli* cells utilizing magnetic silica beads. This kit is based on binding properties of DNA to a silica surface in the presence of chaotropic agents[1]. The purified plasmid can be used directly for automated fluorescent sequencing.

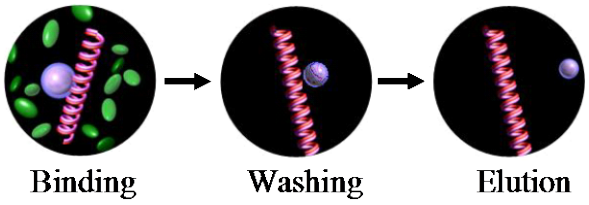

Fig. 1 Principle of purification

Features

-Typical plasmid yield from an *E. coli* cell line carrying a high-copy plasmid is approx. 3-6 µg.
-This kit is suitable for high throughput extraction of plasmid from *E. coli* cells. The extraction time is 10-15 minutes.
-Purified plasmid can be applied directly to sequencing, enzyme reaction, transformation, *etc*.
-This kit does not contain hazardous substances, such as phenol or chloroform.

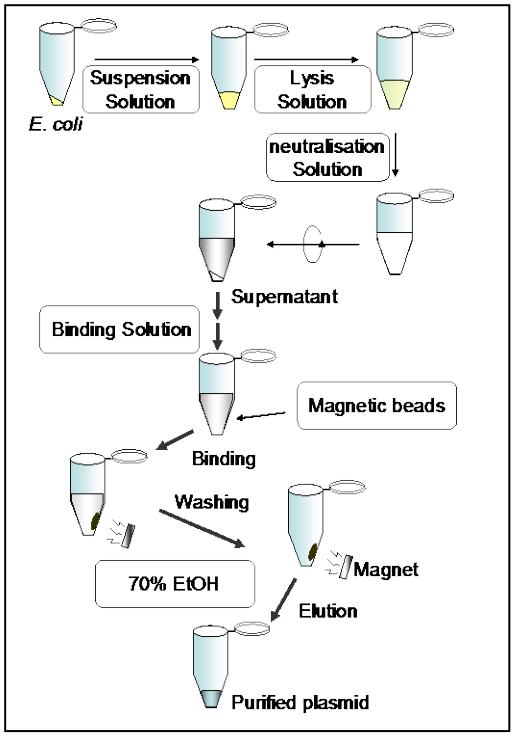

Fig. 2 Flow chart of purification

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[2] Components

This kit contains the following components for 500 preparations.

Binding Solution	130 mL x 2	(store at 4°C or room temperature)
Magnetic Beads I	16 mL	(store at 4°C or room temperature)
Magnetic Beads II	16 mL	(store at 4°C or room temperature)
Suspension Solution	80 mL	(store at 4°C)
Lysis Solution I	80 mL	(store at 4°C or room temperature)
Lysis Solution II	20 mL	(store at 4°C)
Neutralization Solution	65 mL	(store at 4°C or room temperature)
Elution Solution	60 mL	(store at 4°C)
5x Loading Dye	11 mL	(store at 4°C)

Caution

-"**Binding Solution**" contains chaotropic salt, which is an irritant. Lysis solution II contains NaOH. Take appropriate laboratory safety measures and wear gloves when handling.
-"**Suspension Solution**" contains RNase A.

Notes

-**Magnetic Beads I** are not necessary for manual use.
[This reagent is used for extraction with the automated nucleic acid purification apparatus "MFX series"]
-**Lysis solution I** and **II** should be mixed at a ratio of 4:1 prior to use. This solution can be stored at room temperature for 3 weeks.
-If precipitates are formed in "**Lysis Solution I**" at low temperature, dissolve the precipitates by heating at 40°C.
-Purified plasmid may contain small amounts of EtOH. Plasmid DNA solution containing EtOH sink easily into agarose gel slots using **5x Loading Dye.**

[3] Materials required

The following materials are required for purification.

(1) Reagents
-70% Ethanol

(2) Instruments
-Vortex mixer
-Magnetic stand
-(Heating block)

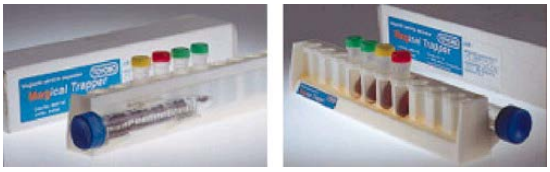

Fig.3 Magnetic stand
Magical Trapper (Code No.MGS-101)

Notes

-For complete evaporation of ethanol, a heating block at 78°C is necessary.

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[4] Protocol

1. Preparation of reagents required

Lysis solution I and **II** should be mixed at a ratio of 4:1 prior to use. This solution can be stored at room temperature for 3 weeks.

2. Purification

(1) Pellet 1.5-3 mL bacterial culture by centrifugation; discard as much supernatant as possible.

Notes
Up to 3 mL of bacterial cultures can be used for high and low copy number plasmids. Bacterial cultures should be incubated for 12-16 hours in appropriate medium containing selection antibiotics.

(2) Resuspend pelleted bacterial cells thoroughly in 150 µL **Suspension Solution**.

(3) Add 150 µL **Lysis Solution (mixed)**, and mix by inverting the tube 5 times (DO NOT VORTEX)

(4) Incubate on ice for 5 minutes.

(5) Add 120 µL l **Neutralization Solution**, and mix by inverting the tube 5 times (DO NOT VORTEX)

(6) Centrifuge at 12,000 rpm for 5 minutes

(7) Carefully transfer all lysate to a fresh 1.5-mL microtube.

(8) Add 500 µL **Binding Solution**.

(9) <**Binding**> Add 30 µL **Magnetic Beads II** and vortex the tube for 1 minute.

Notes
Suspend magnetic beads completely prior to use.

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

Fig. 4
Magnetic separation

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

(12) <**Washing**> Add 720 µL **70% EtOH** to the tube and vortex for 10 seconds.

(13) Place the tube in the magnetic stand and collect the beads with the magnet.

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

(15) <**Washing**> Repeat (12) - (14)

(16) **optional** <**Drying**> Evaporate EtOH by heating the opened microtube to 78°C for ≤ 15 minutes.

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(17) **<Elution>** Add 50 µL **Elution Solution** and vortex for 1 minute.

(18) Collect the supernatant and place in a fresh tube.

Notes:

-Purified plasmid solutions that have not been heated contain small amounts of EtOH. Plasmid DNA solution containing EtOH easily sink into agarose gel slots by using **5x Loading Dye.**

[5] Troubleshooting

Symptom	Cause	Solution
Low yield	Insufficient lysis of *E. coli* cells	Insufficient lysis of *E. coli* cells decreases plasmid yields.
	Low-copy plasmids	Increase the number of *E. coli* cells for purification. When using low-copy plasmids, yields will be low.
Degradation of purified plasmid	Residual DNase	Use DNase-gene deficient *E. coli* strains (*e.g.*, JM109, DH5α, and XL1-Blue). Plasmids from *E. coli* strain carrying DNase-gene (*e.g.*, HB101) might be degraded during incubation.
Unnecessary magnetic beads	Magnetic Beads I	Magnetic Beads I are not necessary for manual use. This reagent is used in extraction with the automated nucleic acid purification apparatus “MFX series”.
Diffusion of DNA solution in electrophoresis buffer	Trace amounts of EtOH in the sample	Plasmid solutions purified without a drying step ight contain small amounts of EtOH. Plasmid DNA solutions containing EtOH easily sink into agarose gel slots by using **5x Loading Dye.**

[6] References

1) B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. USA.* 76: 615-619 (1979)

[7] Related products

Product name	Package	Code No.
Magnetic stand **Mag*ical Trapper***	1 piece	MGS-101

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