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F0986K

MagExtractor-*PCR & Gel Clean up-*

NPK-601 200 preparations

Store at room temperature

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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-PCR & Gel Clean up- provides a simple and reliable method for the rapid purification of DNA fragments from a PCR solution, enzyme solution, or agarose gel slices 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 DNA fragments can be used directly for general molecular biology experiments.

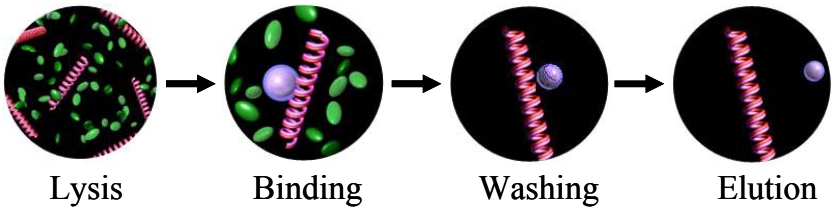


Fig. 1 Principle of purification

Features

-This kit extracts DNA fragments from ≤ 100 µl PCR solutions or enzyme solutions (*e.g.*, restriction enzyme and alkaline phosphatase) within 5 minutes.
-This kit extracts DNA fragment from ≤ 0.3 g agarose gel slices (TAE or TBE) within 15 minutes. Agarose slices can be melted at room temperature; it is not necessary to use a heating block.
-Typical yields from solution or gel slices are approx. 60-70%. DNA fragments approx. 100 bp to 50 kb can be effectively recovered. Small fragments (< 40 bp) can be removed.
-Purified DNA fragments can be applied to sequencing, restriction enzyme treatment, labeling, ligation, transformation, *etc*. RI-labeled probes can also be purified with this kit.

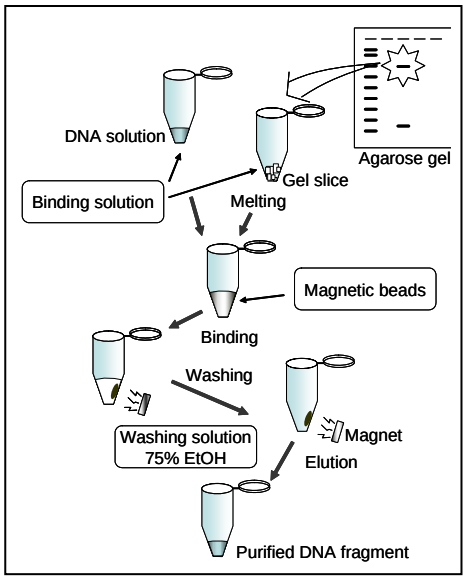


Fig. 2 Flow chart of purification

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[2] Components

This kit contains the following components for 200 preparations. All reagents should be stored at room temperature.

Binding Solution	88 ml
Washing Solution	132 ml
Magnetic Beads	8.5 ml

Caution:
-The "Lysis & Binding" and "Washing" solutions contain chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling.

Notes:
-All reagents should be used at room temperature for extraction.
-If precipitates are present in the "Binding Solution" or "Washing Solution" at low temperatures, dissolve the precipitates by warming in the hand.

[3] Materials required

The following materials are required for purification.

(1) Reagents
-Sterilized water
-75% 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 set at 55°C is necessary.

[4] Protocol

1. Purification from DNA solution. <Standard protocol>

(1) Dispense ≤ 100 µl of DNA solution into a 1.5-ml microtube.

(2) Add 400 µl **Binding Solution**.

(3) **[Binding]** Add 30µl **Magnetic Beads** and vortex the tube every 10 seconds for 1-2 minutes.

Notes
Completely resuspend the magnetic beads prior to use.

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

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



Fig. 4
Magnetic separation

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(6) optional **[Washing]** Add 600 µl **Washing Solution** to the beads and vortex for 10 seconds.

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

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

(9) **[Washing]** Add 1 ml **75% EtOH** to the tube and vortex for 10 seconds.

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

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

Notes
The 75% EtOH should be completely removed after a flash centrifugation.

(12) optional **[Washing]** Repeat (9) - (11)

(13) optional **[Drying]** Evaporate 75% EtOH by heating the opened microtube at 55°C for 5 minutes.

(14) **<Elution>** Add 25-100 µl **sterilized water** and mix well for 10 seconds.

(15) Incubate at room temperature for 2 minutes.

(16) Place the tube in the magnetic stand after briefly vortexing.

(17) Collect the supernatant and place into a fresh tube.

Notes
-Magnetic beads can bind maximum 2.5-5 µg per 30 µl beads.
-The amount of Binding Solution, Washing Solution, and Magnetic Beads can be decreased, depending on the volumes of DNA solutions.
-The following table summarizes the optional steps:

Applications of purified DNA fragments	Wash with		Dry	Remarks
	Washing Solution	75% EtOH		
	X times.			
-Sequencing -Enzyme reactions -Ligation	-	1	-	Fast protocol
-Salt-sensitive reactions	-	2	-	Binding and Washing Solution contains high concentration of salts.
-EtOH-sensitive reactions	(1)	1 or 2	55°C for 5 minutes	EtOH can be evaporated by heating.
-Alkaline phosphatase -sensitive reactions	1	1 or 2	-	Enzymes can be completely removed by washing with Washing Solution.
-Accurate measurement of DNA concentration	1	2	-	Binding Solution contains a substance that absorbs UV.

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2. Purification from agarose gel slices.

(1) While under UV illumination, excise an agarose block (≤ 0.3g) that contains the DNA band of interest using a razor blade or scalpel. TBE or TAE can be used for agarose gels.

Notes
Long wavelength and/or low-power UV illumination should be used for excising gel blocks. If using a transilluminator, gels should be placed on an acrylic plate to prevent strong UV damage.

(2) Slice the agarose block into smaller pieces and place the gel slices into 1.5-ml microtubes.

(3) Add 400 µl Binding Solution.

(4) Incubate at room temperature until gel pieces are completely dissolved. To increase efficiency , vortex the tube every 2-3 min during incubation.

(5) **[Binding]** Add 30µl **Magnetic Beads** and vortex the tube every 10 seconds for 2 minutes.

Notes
Completely resuspend the magnetic beads prior to use.

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

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

(8) **[Washing]** Add 600 µl **Washing Solution** to the beads and vortex for 10 seconds.

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

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

(11) Perform the purification steps (9)-(17) in **Standard protocol.**

Notes
-The amount of Binding Solution, Washing Solution, and Magnetic Beads can be decreased, depending on the volumes of DNA solutions.
-The following table summarizes the optional steps.

Applications of purified DNA fragments	**Wash with**		**Dry**	**Remarks**
	Washing Solution	**75% EtOH**		
	X times.			
-Enzyme reactions -Ligation	1	1	-	Fast protocol
-Salt-sensitive reactions	1	2	-	Binding and Washing Solution contains high concentration of salts.
-EtOH-sensitive reactions	1	1 or 2	55°C for 5 minutes	EtOH can be evaporated by heating.
-Accurate measurement of DNA concentration	1	2	-	Binding Solution contains a substance absorbs UV.

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[5] Troubleshooting

Symptom	Cause	Solution
Low yield	Insufficient removal of EtOH	Residual EtOH might decrease the yield of DNA fragments. After the final washing step, EtOH should be carefully removed.
	Insufficient elution	Elution with 10 mM Tris-HCl (pH8.0) or TE buffer might increase the yield of DNA fragments. Heating at 55°C might also increase yield.
	Insufficient binding time	Prolong the binding time to up to 2 minutes.
	Insufficient suspension when eluting	Insufficient suspension of magnetic beads when eluting decreases the yield of DNA fragments. Resuspend the magnetic beads completely when eluting.
	Too much agarose gel	Excess agarose gel decreases yield. Decrease the amount of agarose gel to 0.3 mg for extraction.
	High agarose concentration (> 2%)	Excess agarose decreases yield. Decrease the amount of agarose gel for extraction.
	Insufficient washing with Washing Solution	The washing step with Washing Solution is necessary when extracting DNA fragments from an agarose gel.
Absorption at 260 nm is too high.	Insufficient washing	Binding Solution contains a substance that absorbs UV.
	Carry-over of Binding Solution	Binding Solution contains a substance that absorbs UV. The Binding Solution, attached to the lid of a microtube, should be carefully removed.
Purified DNA fragment does not work	Inhibition by salts	Increase the washing step to 75% EtOH. Binding and Washing Solutions contain high concentrations of salts.
	EtOH inhibits the reaction	Evaporate EtOH by heating at 55°C for 5 minutes.
	Insufficient removal of enzymes	Washing step with Washing Solution is efficient for removal of enzymes.
	Insufficient enzyme for reaction	Recovered DNA solution from an agarose gel might contain enzyme inhibitors. Add excess amount of enzymes for the DNA fragments.
	The quality (grade) of the agarose is low.	Use high-grade agarose.
	Too much UV damage due to long exposure during gel excision.	Long wavelength and/or low-power UV light should be used for gel block excision. If using a transilluminator, gels should be excised on an acrylic plate to prevent UV damage.

[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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