COSMO BIO CO., LTD.
Inspiration for Life Science

TOYOBO

DNA Fragment Purification Kit

MagExtractor *-PCR & Gel Clean up-*

Instruction Manual

(Code No.NPK-601)

Distributor

COSMO BIO CO., LTD.
Inspiration for Life Science

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618

Contents	Page

Caution

All the reagents included in this kit are for experimental use and are never to be used for diagnostical or clinical purposes. When using this kit, make sure to follow common laboratory safety procedures carefully.

This kit also contains reagents that are harmful to humans. We ask that you wear protective gear and proceed closely following the cautionary items included with each reagent respectively.

1. Introduction

MagExtractor *-PCR & Gel Clean up-* is a manual DNA fragment purification kit that involves nucleic acid absorption properties[1)] in silica in the presence of chaotropic agents. This kit uses magnetized silica particles and, by utilizing a magnetic stand, complex centrifuging is reduced to a bare minimum enabling a quick DNA fragment purification.

1) *Proc. Natl. Acad. Sci. USA* 76 (2) 615-619 (1979)

Features

- An average DNA fragment (approx. 100bp to 50kbp) yield of 60 to 70% can be collected with DNA solution (approx. 100μl) and TAE-TBE agarose gel (approx. 0.3g).
- DNA fragments can be collected in roughly 5 minutes with DNA solution and in less than 15 minutes with agarose gel.
- Because agarose gel dissolves at room temperature, a heat block is not required.*

*For completely removing the ethanol mixed into the collected solution, a heat block (55°C) is necessary.

Applications

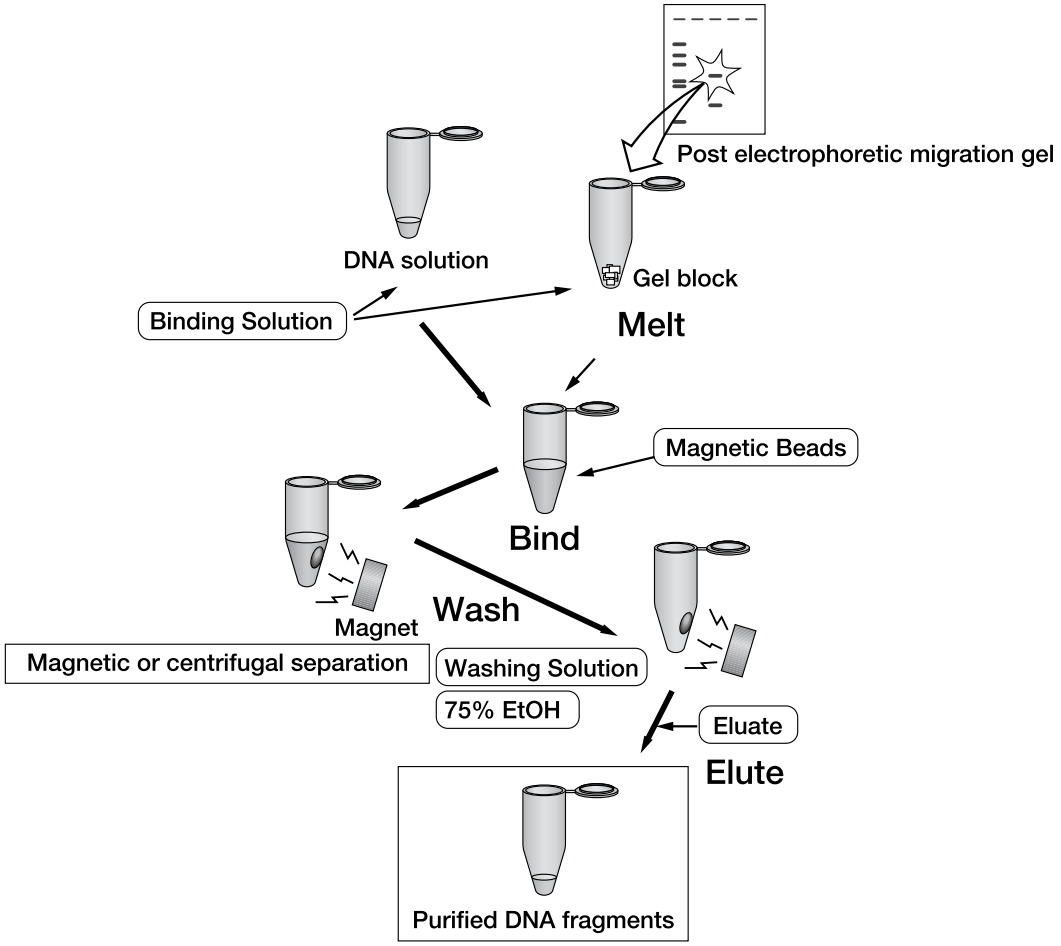
- Desalination, dNTP removal
- Primer removal
- Enzyme removal (alkali phosphatase, etc.)
- Post electrophoretic migration DNA collection from agarose gel block

Applications for the collected DNA

- RI and Non-RI sequencing
- Controlled enzymatic reaction
- Labeling
- Hybridization
- Ligation
- Genetic transformation
- Amplification reaction

2. Purification flow

Below is a description of the purification flow using MagExtractor *-PCR & Gel Clean up-* .



3. Items included in this kit

The following reagents are included in this kit. (200 batches)

	Reagent volume	Storage condition
Binding Solution*	88ml	Store at room temperature shielded from light.
Washing Solution*	132ml	Store at room temperature.
Magnetic Beads	8.5ml	Store at room temperature.

*Having the adsorption or cleansing solution at low temperatures may result in crystal precipitation. Use them after dissolving the crystals by gently tumble mixing manually. Also, the adsorption and cleansing solutions contain protein denaturant. Exercise caution when handling and immediately rinse away and clean off any solution that may get on the body.

4. Required items not included in this kit

[1] Reagents

- Sterilized distilled water <eluate>
- 75% ethanol <for cleansing>

[2] Devices and equipment

- 1.5ml micro-tube magnetic stand*
- Simple tabletop centrifuge
- Vortex mixer
- 55°C heat block (for when completely removing the ethanol from the collected solution)

*Our Mag*ical Trapper* (Code No.: MGS-101) magnetic stand may be used.

5. Collection from DNA solution

[1] Introduction

- The protocols for this kit are used for the collection of DNA fragments from a DNA solution (approx. 100μl).
- Collectable DNA sizes range from roughly 100bp to 50kb. Also, nucleic acid that is 40bp or less is mostly excluded.
- The maximum adsorption amount for magnetic beads is approx. 5μg for each 30μl bead. Use 2.5μg as a standard guideline.
- DNA collection is possible from various reaction solutions including PCR reaction solutions, controlled enzymatic reaction solutions, alkali phosphatase reaction solutions, etc.
- The collected DNA can be used for many reactions such as controlled enzymatic treatments, sequencing, ligation, labeling, hybridization, etc.

[2] Protocol

DNA solution (100μl)*1

↓ ←400μl Binding Solution
←30μl Magnetic Beads*2
Stir at times with a vortex, leave for roughly 1 to 2 minutes (at room temperature)

B/F (solid/liquid) separation*3

↓ ←(600μl Washing Solution)*4
Stir for 10 seconds with a vortex mixer.

B/F separation*3

↓ ←1ml 75% Ethanol*4
Stir for 10 seconds with a vortex mixer.

B/F separation*3
Spin down and completely remove the supernatant.
(Open the microtube lid and leave for 5 minutes at 55°C: dry)*4

↓ ←25 up to 30μl distilled water
Stir for 10 seconds with a vortex mixer.

(Should the particles not sufficiently loosen, perform pipetting to loosen them.)

↓ Leave for 2 minutes

B/F separation, supernatant collection*5

*1. It is preferred not to introduce mineral oil.

*2. Suspend the solution well prior to using the magnetic beads.

*3. By setting the tubes onto the magnetic stand, the magnetic beads are drawn to the magnet. Remove at this time the supernatant with the pipette. If cleansing with ethanol, you may remove the supernatant by decantation.
For B/F separation, using a magnetic stand especially for 1.5ml microtubes is recommended. However, the same operation may also be performed using a simple tabletop centrifuge at 6,000 rpm for 5 seconds. The "g" can be changed with the centrifuge's rotor diameter. Adjust the rotational frequency to enable an efficient particle collection and to minimize coagulation.

*4. See the [3] table on next page.

*5. Having some magnetic beads remaining in the collected solution will not inhibit the proceeding reaction. However, if determining light absorption, remove by performing spin down.

[3] Process abridgement and purification scale

·It is possible to abridge the cleansing and heating processes (see the table below).

Application	Cleansing		Drying	Remarks
	Washing Solution	75% ethanol		
Sequencing, controlled enzymatic reaction, ligation pretreatment	——	Once	——	This is standard protocol. The collected nucleic acid can also be used for cutting off with the controlled enzymes of low buffer systems.
If the introduction of salt affects the reaction	——	Twice	——	The adsorption and cleansing solutions have a high concentration of salt.
If the introduction of ethanol affects the reaction	——	Once (twice)	55°C, 5 min.	Used during pretreatment of reactions easily affected by ethanol.
When complete and rigorous removal of enzymes is desired	Once	Once (twice)	——	Used to remove alkali phosphatase.
When accurate measurement of nucleic acid concentration by light absorption is desired	Once	Twice	——	The adsorption solution contains substances with absorbed ultraviolet rays.

·Depending on the volume of DNA solution, it is possible to proportionally decrease the amount of Binding & Washing Solution as well as Magnetic Beads included in this kit. When doing so, it is not necessary to lower the 75% ethanol amount.

6. Collection from agarose gel

[1] Introduction

- This kit's protocols are used for the collection of DNA fragments from TAE or TBE agarose gel (approx. 0.3g) and are intended for a gel with an agarose concentration of no more than 2%. If using a gel with 2% or more, making the gel amount 0.3g or less is recommended. Also, it is not necessary to use agarose of a low melting point.
- Collectable DNA sizes are approx. from 100bp to 50kb.
- Collected DNA can be mainly used for ligation, labeling, sequencing or other reactions.

[2] Protocol

Agarose gel electrophoretic migration, ethidium bromide staining

↓

Under UV irradiation (long wave), it is preferable to make the target bands smaller by cutting using a cutter knife or scalpel.

↓

Finely slice the cut out agarose and insert it in the 1.5ml microtube.
(At this time, measure weight and divide the sample if over 0.3g.)*1

↓ ←400μl Binding Solution

Leave at room temperature and stir occasionally until the gel is completely dissolved.*2

↓ ←30μl Magnetic Beads*3
Leave for 2 minutes (at room temperature) and stir occasionally with a vortex.

B/F separation*4

↓ ←600μl Washing Solution*4
Stir for 10 seconds with a vortex mixer.

B/F separation*4

↓ ←1ml 75% Ethanol*5
Stir for 10 seconds with a vortex mixer.

B/F separation*4
Spin down and completely remove the supernatant.
(Open the microtube lid and leave for 5 minutes at 55°C: dry)*5

↓ ←25 up to 30μl distilled water
Stir for 10 seconds with a vortex mixer.

(Should the particles not sufficiently loosen, perform pipetting to loosen them.)

↓ Leave for 2 minutes

B/F separation, supernatant collection*6

*1. Agarose gel dissolution is made easy by slicing it very finely.

*2. If the agarose gel is difficult to dissolve or if a quick dissolution is desired, apply 55°C of heat for 5 minutes.

*3. Suspend the solution well prior to using the magnetic beads.

*4. By setting the tubes onto the magnetic stand, the magnetic beads are drawn to the magnet. Remove at this time the supernatant with the pipette. If cleansing with ethanol, you may remove the supernatant by decantation.
For B/F separation, using a magnetic stand especially for 1.5ml microtubes is recommended. However, the same operation may also be performed using a simple tabletop centrifuge at 6,000 rpm for 5 seconds. The "g" can be changed with the centrifuge's rotor diameter. Adjust the rotational frequency to enable an efficient particle collection and to minimize coagulation.

*5. See the [3] table on next page.

*6. Having some magnetic beads remaining in the collected solution will not inhibit the proceeding reaction. However, if determining light absorption, remove by performing spin down.

[3] Process abridgement and purification scale

・It is possible to abridge the cleansing and heating processes (see the table below).

Application	Cleansing		Drying	Remarks
	Washing Solution*	75% ethanol		
Sequencing, ligation	Once	Once	——	This is standard protocol.
If the introduction of salt affects the reaction	Once	twice	——	The adsorption and cleansing solutions have a high concentration of salt.
If the introduction of ethanol affects the reaction	Once	Once (twice)	55°C, 5 min.	Used during pretreatment of reactions easily affected by ethanol.
When accurate measurement of nucleic acid concentration by light absorption is desired	Once	twice	——	The adsorption solution contains substances with absorbed ultraviolet rays.

*If collecting nucleic acid from agarose gel, cleansing with Washing Solution is necessary.

・Depending on the amount of agarose gel, it is possible to proportionally decrease the amount of Binding & Washing Solution as well as Magnetic Beads included in this kit. When doing so, it is not necessary to lower the 75% ethanol amount.

7. Trouble shooting

[1] Low yield

Cause	Remedy
Incomplete ethanol removal	After spin down, remove the 75% ethanol with the utmost caution.
Insufficient dissolution	Dissolution rate may increase by using 10mM Tris-HCI (pH 8.0) or TE buffer. Also, the dissolution rate may be increased even further by applying 55°C of heat for 2 minutes.
Inappropriate adsorption time span	Excessive adsorption may cause low yield. Optimal adsorption time is 1 to 2 minutes for DNA solution and approx. 2 minutes for gel.
Insufficient stirring during dissolution	If the magnetic beads are insufficiently suspended during dissolution, the resulting yield may be low. After spin down, the beads may get stuck on the bottom. Make sure to loosen them up from the bottom. The beads are especially difficult to disperse when purifying using agarose gel.
Agarose gel amount exceeds 0.3g	Reduce the agarose gel amount.
2% or more agarose concentration is in use	Reduce the agarose gel amount.
Cleansing is not performed with Washing Solution	When collecting DNA from agarose gel, make sure to perform cleansing using Washing Solution.

[2] Inaccurate measured quantity of collected nucleic acid as determined by light absorption rate

Cause	Remedy
Insufficient cleansing	The Binding Solution contains substances with absorbed ultraviolet rays. If measuring the collected nucleic acid quantity, perform cleansing with Washing Solution and with 75% ethanol.
Binding Solution incorporated	The Binding Solution contains substances with absorbed ultraviolet rays. Remove the Binding Solution with the utmost caution. Improve results further by using a tissue for wiping away clean areas such as the tube caps that have drops of Binding Solution still adhering.

[3] Unfavorable reaction using collected nucleic acid

Cause	Remedy
Salt is inhibiting the reaction	Perform 75% ethanol cleansing twice. The Binding and Washing Solutions contain a high concentration of salt.
Ethanol is inhibiting the reaction	Perform the reaction closely following the ethanol drying protocols.
Enzymes not completely removed	For the complete removal of enzymes such as alkali phosphatase from the reaction solution, perform cleansing with cleansing solution as well as 75% ethanol.
Insufficient enzymes used in the reaction	If using nucleic acid collected from agarose gel, favorable results may be obtained by setting the concentration of enzymes used in the reaction higher than normal.
Low agarose grade	Use high grade agarose.
Overexposure of ultraviolet rays when cutting bands from the agar-	Perform cutting along the long wave (near 365nm) ultraviolet rays.

Manufacture
TOYOBO
TOYOBO CO., LTD.
Life Science Department
2-8,Dojima Hama 2-chome, Kita-ku, Osaka 530-8230, JAPAN

Distributor
COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp
e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617
FAX : +81-3-5632-9618