COSMO BIO CO., LTD.
Inspiration for Life Science

TOYOBO

Nucleic Acid Purification Kit

MagExtractor *-Plasmid-*

Instruction Manual

(Code No.NPK-301)

Distributor

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Inspiration for Life Science

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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.

1. Introduction

MagExtractor -*Plasmid*- performs plasmid DNA extraction and purification utilizing the adsorption action of nucleic acid onto the surface of silica in the presence of chaotropic agents. Magnetic material enclosed beads enable easy separation and collection of nucleic acid using a permanent magnet. The adsorption capacity of these magnetic beads for protein, DNA or RNA varies depending on the adsorption conditions, therefore, the target nucleic acid can be purified with a simple operation. Aside for use as a reagent for the MFX-2100 fully automatic nucleic acid extractor, this kit can also be used with the manual extraction method.

Capabilities and features

・Plasmid DNA can be extracted and purified from *E.coli*.
・3 to 6μl of plasmid DNA (50 to 150ng/μl) can be collected*1 and used for genetic transformation, restriction enzyme treatment or DNA sequencing reactions.
・Plasmid DNA can be extracted in 10 to 15 minutes.
・Does not use harmful solvents such as phenol or chloroform.
・Ethanol precipitation is not required.

2. Cautions prior to use

[1] Caution

MagExtractor -*Plasmid*- has 4 types of protocols for use with the MFX-2100 and 2 types for manual operation. Below are cautionary items for the solution collected under the protocol that does not allow the magnetic beads to dry (manual protocol I and the "Speedy" and "Lowcost" protocols for the MFX-2100).

・Use as is for restriction enzyme treatment or genetic transformation
・Make sure to use the loading dye included with this kit when performing agarose electrophoretic migration. With normal composition, the sample may not settle down in the well.
・If using as a template for DNA sequencing, ethanol will inhibit the sequencing reaction therefore insert the collected solution in a 1.5ml tube, leave the lid open and apply 78℃ for 40 minutes (heat treatment can be done for only the amount necessary. See P.10).
・To prevent the carry over of the ethanol in the collected solution, use manual protocol II (see P.10) or MFX-2100 protocols "Dryup" or "Fullauto".

*1. If extracting from a JM109/pUC19 overnight culture (5 to 8 O.D.).

3. Items included in this kit

There are enough reagents in this kit to perform 500 plasmid DNA extractions.

130ml×2 --- Adsorption Solution (with protein denaturant) -------4℃ or room temperature
20ml -----Magnetic Beads I ----------4℃ or room temperature
20ml -----Magnetic Beads II----------4℃ or room temperature
80ml -----Suspension Solution ----------4℃
80ml -----Lysis Solution I (with protein denaturant) ----------4℃ or room temperature
20ml -----Lysis Solution II (low concentration buffer) ----------4℃
65ml -----Neutralizing solution ----------4℃ or room temperature
10ml -----Elution Solution ----------4℃
11ml -----5×Loading Dye ----------4℃

・Should some of the reagents get on the hands or clothing, make sure to wash them out thoroughly. In the likely event that some should get in the eyes, wash them out well and consult a physician.
・There may be precipitants forming out of Lysis Solution I. Completely dissolve between room temperature and 40°C prior to use.
・Store the mixture of Lysis Solution I and II at room temperature and do not use if over 3 weeks of storage has elapsed.*1 For all other reagents, store at 4°C.
・If using our MFX-2100 automated nucleic acid extractor, dispense the amount needed in the designated bottles and set in the predetermined positions. For details, see the MFX-2100 instruction manual.

*1. The yield and purity of plasmid DNA may decrease.

4. Protocol

[1] Required items not included in this kit

(1) Reagents

・Sterilized water (distilled water or autoclave sterilized mili-Q water)
・Ethanol (high-grade 99.5% proof, 70% ethanol for manual)

(2) Devices and equipments

・Micropipette
・Micropipette chips

When using the MFX-2100:

・Extraction specialized tubes (Code No.: MFX-301 and MFX-302) for sample collection
・Chip with special filter attached (Code No.: MFX-402A)
・Reagent setting tubes (50ml, 15ml, 2ml *1)

For manual extraction

・1.5ml micro-tube magnetic stand (commercially available)
・Vortex mixer (micro-tube mixer)
・Simple tabletop centrifuge (12,000 r.p.m.)

*1. For 50ml or 15ml tubes, use Becton Dickinson's Blue Max 2170 or 2196 and Assist's No.72.693 or No.72.694 for 2ml tubes.

[2] Extraction flow

MagExtractor *-Plasmid-* requires the following 5 processes to enable plasmid DNA extraction and purification.

(1) Harvesting
(2) Alkalilysis
(3) Neutralization
(4) Bacteria residue removal using magnetic beads I
(5) Plasmid DNA separation and purification using magnetic beads II
Using the MFX-2100 with MagExtractor *-Plasmid-* enables the automation of steps (2) to (5).

[3] Host bacteria culture

Incubate plasmid DNA containing colon bacilli. The same article used with LB or TB media and with common methods such as alkali SDS can be used for plasmid DNA extraction. The plasmid yield is affected by copy count and bacteria amount, therefore, using the MMI culture medium, which can ensure a more stable yield with MagExtractor *-Plasmid-*, is recommended. If using the MMI medium, the bacteria amount is equal to the TB medium (JM109/pUC18, 50μg/ml ampicillin 9 to 11 O.D.).

MMI medium preparation method

←Distilled water 800ml
←13g Bacto trypton
←25g Bacto yeast extract
←8.5g Sodium chloride
←4ml glycerol
←20ml 1M Tris-HCI, pH 7.2
Make for 1000ml of distilled water
Perform autoclave

[4] Harvesting

With MagExtractor *-Plasmid-*, the treatable bacteria amount with manual extraction is 12 to 13 O.D. and 5 to 9 O.D. using the MFX-2100. Figure 1 shows yield variations with respect to bacteria amount and extraction method. Treating over 10 O.D. with the MFX-2100 makes for insufficient mixing (pipetting) which may result in a decrease in yield and purity. Use an angle rotor and refer to Figure 2 as a guideline for bacteria amount if harvesting in 1.5ml tubes.

・Insert the culture solution into 1.5ml tubes.
・Perform centrifugal separation at 12,000rpm for 2 minutes.
・Remove the supernatant carefully.

Fig. 1: Yield variation as per bacteria amount (JM109/pUC18, MMI culture)

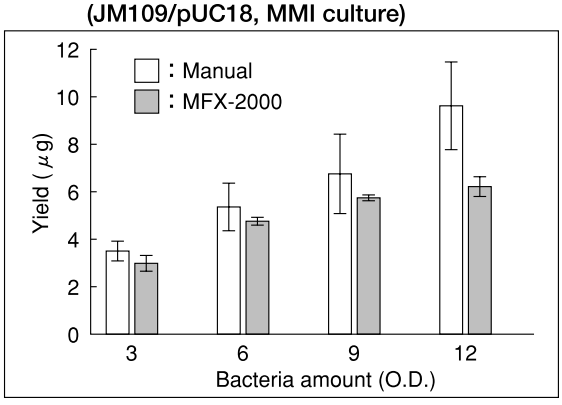
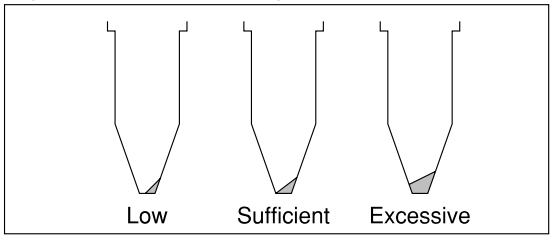


Fig. 2: Bacteria amount guideline.



[5] Reagent preparation

Lysis Solution

Mix Lysis Solutions I*1 and II included with this kit at a 4:1 ratio (v:v) respectively. Store this mixture at room temperature and do not use if storage period exceeds one month.*2 For manual extraction, 150μl per sample is necessary. If using the MFX-2100, prepare referring to the table on P.9 "Guideline for dispensing amount for each reagent".-70% ethanol

Required only for manual extraction. 1500μl/sample is necessary.

*1 If there appears precipitates, dissolve completely between room temperature and 40°C prior to use.
*2 Plasmid DNA yield and purity may drop.

[6] Plasmid DNA extraction using the MFX-2100

Before using the MFX-2100, make sure to read its operation manual thoroughly.
If using "Dryup" or "Fullauto" of the preprogrammed extraction protocols, an MFX-2100 model with a heating function is required.

(1) Selecting protocols

Four plasmid DNA extraction protocols for the MFX-2100 have been prepared. Select one protocol and enter its corresponding number in the liquid crystal display.

・Only one protocol can be selected for one experiment.
・Reagent dispensing time is not included in the extraction times described below. The actual extraction time varies depending on the sample count, however, use "[extraction time listed below] × [sample count] × [1.25 (time)] = actual time needed" as a reference equation.

No.	Description appearing in the 3rd row of the display panel	Sample	Extraction Time	Application[1]	
				A	B
31	[Plasmid:Speedy]	Resuspension	10 min	○	△[2]
32	[Plasmid:Dryup]	Resuspension	15 min	○	○
33	[Plasmid:Fullauto]	Harvested bacteria	16 min	○	○
34	[Plasmid:Lowcost]	Bacteria extract	6 min	○	△

1 : Application A: Restriction enzyme treatment, transformation
Application B: DNA sequencing, or a reaction carrying over 50% or more of the collected solution
2 : DNA sequencing possible if performing manual ethanol removal.

Protocol "31: Speedy"

Suitable for preparing plasmid DNA for restriction enzyme treatment and genetic transformation. Set the resuspension solution in the MFX-2100 and extract the plasmid after approx. 10 minutes per sample.

Protocols "32: Dryup" and "33: Fullauto"

Suitable for preparing plasmid DNA for DNA sequencing. "32: Dryup" is suitable for setting a resuspension solution for treatment of a comparably large culture (7 to 9 O.D.). "33: Fullauto" is suitable for setting bacteria harvested from a comparably small culture (5 to 6 O.D.).

Protocol "34: Lowcost"

Sets samples treated using the following operations. Only 1 special chip is used per sample (3 to 4 for other protocols) enabling a low cost plasmid DNA collection. Plasmid DNA can be prepared for restriction enzyme treatment or transformation in 6 minutes per sample.

Harvested bacteria

↓

←150μl resuspension solution
Agitate for 60 seconds with a vortex
←150μl Lysis Solution (4:1 mixture of Lysis Solution I and II)
Tumble stir tubes 5 times
Put on ice for 5 minutes
←120μl Neutralizing Solution
Tumble stir tubes 5 times
Put on ice for 5 minutes
12,000 rpm 10 minutes

↓

Set in MFX-2100

(2) Heating block and collection block temperature setting*1

	Heating block	Cooling block
Set temperatur	78℃	10℃

*1 Refer to the MFX-2100 instruction manual for the setting method.

(3) Setting chips with special attached filter

- Use specially made filter attached chips (Code No.: MFX-402A).
- The chips are gamma radiation sterilized. Set the number of chips needed in the chip rack using gloves.
- For the chip setting position, refer to the MFX-2100 instruction manual.
- Set the number of chips necessary into the chip rack.

		Chip count						Chip count			
Protocol No.		31	32	33	34	Protocol No.		31	32	33	34
Sample count	1	10	11	11	5	Sample count	13	61	74	74	26
	2	13	15	15	6		14	64	78	78	27
	3	16	19	19	7		15	67	82	82	28
	4	19	23	23	8		16	70	86	86	29
	5	27	32	32	12		17	78	95	95	33
	6	30	36	36	13		18	81	99	99	34
	7	33	40	40	14		19	84	103	103	35
	8	36	44	44	15		20	87	107	107	36
	9	44	53	53	19		21	95	116	116	40
	10	47	57	57	20		22	98	120	120	41
	11	50	61	61	21		23	101	124	124	42
	12	53	65	65	22		24	104	128	128	43

(4) Setting the special tubes

・Set the special tubes (Code No.: MFX-301 or MFX-302) onto the extraction rack. The setting position varies depending on the protocol used. Refer to the table below and set the tubes accordingly.

Protocol No.	Protocol No.
31 to 33	A, B, C, D, E, F
34	A, B, C, D

・Set the specimens on the heating block (only for protocols 32 and 33) and collection block.
・Do not set tubes in the extraction rack's A to F slots and the heating block other than the specially made tubes referred to above. Doing so may cause problems.
・Use Assist tubes No.72.730 for the collection block.*1

*1 Aside from the tubes specially made for the MFX-2100, screw cap type 1.5ml tubes (Assist, No.72.692) can be set onto the collection block. With these tubes, there may be a certain percentage of magnetic beads remaining in the collected solution. If carrying over 50% or more collected solution, centrifuge at 12,000 rpm for 2 minutes and use the resulting supernatant (there is no need for this if 50% or more of the collected solution is not carried over into the reaction solution).

(5) Setting reagents

・Sterilized water and ethanol (high-grade 99.5% proof or more) are not included in this kit.
・Use the 50ml capacity Falcon 2170, 15ml Falcon 2196, 2ml Assist tube No.72.694.
・The reagent amount needed varies with respect to the sample count. Refer to the table on P.7 and dispense the needed amount of reagent into the specified tubes.
・When dispensing reagents, use the gradients marked on the side of the reagent tubes as a guide.
・There are reagents that need not be set as per the protocol under use.
・Set each tube into the designated positions on the reagent rack. Shake the magnetic beads I and II in advance and set onto the reagent rack. Start the device soon after having set the magnetic beads (within 10 minutes) (Not doing so may cause yield fluctuations and operational malfunctions).
・The reagents remaining after extraction may be reused (adding a reduced amount and setting into the MFX-2100 is possible). Close the lid and store portions not to be used soon after an experiment. Storing solutions for extended periods without closing the lid tightly may result in a change in solution composition.
・Do not set amounts exceeding those prescribed in the table on P.7. Doing so causes soiling of the nozzle and spills.

Reagent dispensing amounts for a sample count of 1 to 12

Reagent	Tube capacity	Dispense amount (ml)				Set position
		Protocol No.				
		31	32	33	34	
Adsorption Solution	50ml	25	25	25	25	1
Sterilized water	50ml	25	25	25	25	2
Ethanol	50ml	25	25	25	25	6
Magnetic Beads Ⅰ	2ml	1.5	1.5	1.5	Not. Req.	7
Magnetic Beads Ⅱ	2ml	1.5	1.5	1.5	1.5	8
Suspension Solution	15ml	Not. Req.	Not. Req.	5	Not. Req.	9
Lysis Solution	15ml	5	5	5	Not. Req.	10
Neutralizing Solution	15ml	5	5	5	Not. Req.	11
Elution Solution	15ml	5	5	5	5	12

Reagent dispensing amounts for a sample count of 13 to 24

Reagent	Tube capacity	Dispense amount (ml)				Set position
		Protocol No.				
		31	32	33	34	
Adsorption Solution	50ml	25	25	25	25	1
Sterilized water	50ml	25	25	25	25	2
Ethanol	50ml	50	50	50	50	6
Magnetic Beads Ⅰ	2ml	1.5	1.5	1.5	Not. Req.	7
Magnetic Beads Ⅱ	2ml	1.5	1.5	1.5	1.5	8
Suspension Solution	15ml	Not. Req.	Not. Req.	5	Not. Req.	9
Lysis Solution	15ml	5	5	5	Not. Req.	10
Neutralizing Solution	15ml	5	5	5	Not. Req.	11
Elution Solution	15ml	5	5	5	5	12

(6) Pre-treatment method for samples and settings

Some protocols require pretreatment of the following samples.

(1) For protocols 31 and 32

Harvested bacteria
↓
←150μl Suspension Solution
Agitate for 60 seconds with a vortex
↓
Set the sample holes

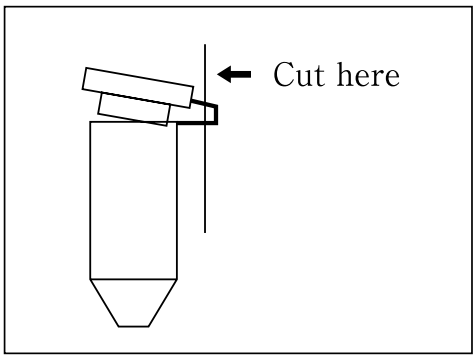
(2) For protocols 33

・Set the harvested bacteria as is in the sample holes.

(3) For protocols 34

Harvested bacteria
↓
←150μl Suspension Solution
Agitate for 60 seconds with a vortex
←150μl Lysis Solution (4:1 mixture of Lysis Solution I and II)
Tumble stir tubes 5 times
Put on ice for 5 minutes
←120μl Neutralizing Solution
Tumble stir tubes 5 times
Put on ice for 5 minutes
12,000 rpm 10 minutes
↓
Set the sample holes*1

・Set the samples into the sample holes with the tube caps off (the front of each hole is marked with a number 1 to 24).
・If using common 1.5ml tubes as a sample tube, cut the caps off with scissors or other means and set. Exercise caution when cutting.



*1 Set the tubes as is even if there is bacteria residue clinging onto the tube walls. The action of the rotor may cause bacteria residue to accumulate on the bottom of the tubes. For such a case, move the supernatant to another tube and set.

(7) Initiate extraction

・Verify whether the samples, reagent tubes (reagent amount), tubes, and special chips are set as per the operation manual.
・Empty the chip disposal box prior to extraction.
・Verify the set position of each rack (lock) and whether the stage is completely contained in the back. Close the front cover.

・Enter the protocol number and the number of samples in the liquid crystal display screen.
・Verify whether "press start key" is displayed on the screen and press "START".
・Extraction initiates and the liquid crystal display screen shows "in operation".
・During operation, hands or other objects may get caught in the device. Never open the front cover while the device is in operation.

(8) Collect samples

・When all extractions are completed, "press start key" will be redisplayed.
・The extracted DNA will collect in the tubes on the collection block. After verifying that the device is completely stopped, remove the tubes from the collection block and put on ice or store at a low temperature (4 to 10℃) until ready to use.
・There may be a small amount of magnetic beads remaining in the collected solution, this will not cause any problems.
・Before removing the tubes from the heating block, turn OFF the heating block switch and check that it is reducing in temperature to under 40℃. Removal while the block is still hot may cause burns.

(9) Manual plasmid DNA extraction method

(1) Protocol I (restriction enzyme treatment and transformation grade)

Harvested bacteria
↓
←150μl Suspension Solution
Agitate for 60 seconds with a vortex
←150μl Lysis Solution (4:1 mixture of Lysis Solution I and II)
Tumble stir tubes 5 times
Put on ice for 5 minutes
←120μl Neutralizing Solution
Tumble stir tubes 5 times
Put on ice for 5 minutes
12,000 rpm 5 minutes
Insert the supernatant into a new 1.5ml tube
←500μl Adsorption Solution
←30μl Magnetic Beads Ⅱ (tumble stirred uniformly mixed)
Agitate for 60 seconds*1
B/F separation*2
←720μl 70% ethanol
Agitate for 30 seconds (repeat twice)
B/F separation
←50μl Elution Solution
Agitate for 60 seconds
12,000 rpm 5 minutes
B/F separation
↓
Supernatant

*1 Agitated at room temperature using a vortex or tube mixer set at maximum speed.
*2 Set the tube onto the magnetic stand and bring the magnet beads towards the magnetic until it has lured out all the beads that adhered to the cover because of the persistent tumble mixing. Afterwards, strongly shake the magnetic stand to let any solution adhering the cover fall out. Extract the supernatant with a pipette.

(2) Protocol Ⅱ (DNA sequencing grade)

Harvested bacteria

←150μl Suspension Solution
Agitate for 60 seconds with a vortex
←150μl Lysis Solution (4:1 mixture of Lysis Solution I and II)
Tumble stir tubes 5 times
Put on ice for 5 minutes
←120μl Neutralizing Solution
Tumble stir tubes 5 times
Put on ice for 5 minutes
12,000 rpm 5 minutes
Insert the supernatant into a new 1.5ml tube
←500μl Adsorption Solution
←30μl Magnetic Beads Ⅱ (tumble stirred uniformly mixed)
Agitate for 60 seconds
B/F separation
←720μl 70% ethanol
Agitate for 30 seconds (repeat twice)
B/F separation
←500μl ethanol
Agitate for 30 seconds
B/F separation
78°C, 15min (dry the magnetic beads)
←50μl Elution Solution
Agitate for 60 seconds
12,000 rpm 5 minutes
B/F separation

Supernatant

5. Analysis of a common sample

[1] A_{260}, A_{280} or A_{320} measurement

・Measure after spinning down the collected solution.
・Measure after having diluted to between 10 and 30 times with a TE buffer solution.

[2] Electrophoresis

・5 × loading dye : sample : Electrophoresis buffer solution for migration= a 2:3:5 to 2:7:1 ratio dilution

[3] DNA sequencing

・Use the DNA extracted under protocols 32 and 33, or manual protocol II, for a 5 to 10μl reaction.
・In the DNA extracted under protocol 31 and 34, or manual protocol I, there is roughly 10% ethanol contamination that will inhibit the sequencing reaction. Remove the ethanol.
* Dispense 15μl of the sample in a 0.5ml tube.
* Apply 78℃ of heat to the solution until it is completely evaporated (about 15minutes) and use this tube to mix the reaction solution.

6. Trouble shooting

[1] When using the MFX-2100

(1) Low plasmid DNA yield and poor level of purity

Cause	Remedy
Insufficient reagent amount	There is 800μl, 720μl and 720μl of reagent remaining in holes C, D and E respectively after having performed B/F separation. If these amounts are low, then the amounts originally set are insufficient. Set the prescribed amounts. In the case of a low amount of collected solution, or none at all, then the eluate amount is low or none was set originally.
Sample amount excessive	If there is 50μl or more solution remaining in the extraction tubes of the A and B holes, then the bacteria amount was excessive and pipetting could not have been performed using the special chips. Treat 9 O.D. or less of culture.

[2] When using the manual method

(1) Low plasmid DNA yield

Cause	Remedy
Insufficient amount of Magnetic Beads II	Use the prescribed amount of magnetic beads II.
Low amount of Adsorption Solution	Use the prescribed amount of Adsorption Solution.
Magnetic beads cleansed with 70% or less ethanol concentration or the cleansing time allotted is too long	Cleanse with 70% ethanol. Do not cleanse for over 1 minute.
Low eluate amount	Use 50μl or more eluate.
Insufficient plasmid DNA copy count	Increase the extraction scale if using the manual method. The bacteria and reagent amounts can be increased to up to double the amount. However, the eluate amount can also be raised without increasing the concentration of collected solution.

(2) Poor plasmid DNA purity

Cause	Remedy
Bacteria dissolving and residue coagulation process inadequate	Regulate the dissolving time and the solvent amount as per the prescribed amounts. If the level of purity is low during this process, then the magnetic beads will not scatter adequately during adsorption-cleansing resulting in a collected solution with a poor level of purity.
Magnetic beads cleansed with 70% or more ethanol concentration or the cleansing time allotted is too short	Cleanse using 70% ethanol. Cleanse until the magnetic beads are completely scattered (1 minute).

Manufacture

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