

- For extraction of DNA fragments from agarose gel
- For purification of PCR products or reaction mixtures (concentration and desalination of reaction mixtures)

## Kit Contents:

Cat. No:	FAEPK 000B (4 preps_sample)	FAEPK 001B (50 preps)	FAEPK 001-1B (100 preps)
MF Buffer	3 ml	30 ml	60 ml
Wash Buffer (concentrate) <sup>a</sup>	1 ml	12.5 ml	20 ml
Elution Buffer	0.5 ml	5 ml	5 ml
MF Columns *	4 pcs	10 x 5 pcs	10 x 10 pcs
Collection Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer <sup>a</sup>	4 ml	50 ml	80 ml

\*Store the MF Columns to 4 ~ 8 °C upon receipt.

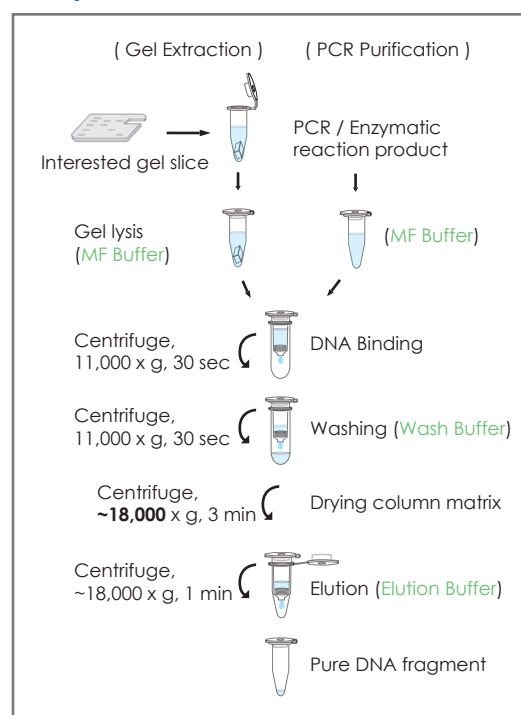
## Specification:

Principle: spin column (silica matrix)  
 DNA Binding capacity of spin column: 5 µg  
 Sample size: up to 200 mg of agarose gel  
 up to 100 µl of reaction solution  
 DNA size: 65 bp ~ 10 kbp  
 Recovery: 70% ~ 85% for Gel extraction  
 85% ~ 95% for PCR clean-up  
 Operation time: 10 ~ 20 min  
 Elution volume: 10 ~ 12 µl

## Important Notes:

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffer.
2. Add the required volume of ethanol (96~100%) to Wash Buffer before use.
3. For gel DNA extraction, excising the extra agarose gel to minimize the size of the gel (up to 200 mg).
4. For concentration or purification of DNA fragments from enzymatic reactions, the maximum sample volume is 100 µl and the maximum amount of DNA fragments is 5 µg.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

## Brief procedure:



## Gel Extraction Protocol: For extraction of DNA fragments from agarose gel

Please Read Important Notes Before Starting Following Steps.

**HINT: Prepare a 55 °C dry bath or water bath for step 4.**

1. **Excise the agarose gel with a clean scalpel.**
  - Remove the extra agarose gel to minimize the size of the gel slice.
2. **Transfer up to 200 mg of the gel slice into a microcentrifuge tube.** (not provided).
  - The maximum volume of the gel slice is 200 mg.
3. **Add 500 µl of MF Buffer to the sample and mix by vortexing.**
  - For > 2% agarose gels, add 1000 µl of MF Buffer.
4. **Incubate at 55 °C for 5 ~10 minutes and vortex the tube every 2 ~ 3 minutes until the gel slice dissolved completely.**
  - During incubation, interval vortexing can accelerate the gel dissolved.
  - Make sure that the gel slice has been dissolved completely before proceed the next step.
5. **Cool down the sample mixture to room temperature. And place a MF Column into a Collection Tube.**
6. **Transfer 700 µl of the sample mixture to the MF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.**
  - If the sample mixture is more than 700 µl, repeat this step for the rest of the sample mixture.
7. **Add 600 µl of Wash Buffer (ethanol added) to the MF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.**
  - Make sure that ethanol (96-100 %) has been added into Wash Buffer when first use.

8. Centrifuge again at full speed (~ 18,000 x g) for an additional 3 minutes to dry the column matrix.
  - **Important step!** The residual liquid should be removed thoroughly on this step.
9. Place the MF Column to a new microcentrifuge tube (not provided).
10. Add 10 ~ 12 µl of Elution Buffer or ddH<sub>2</sub>O to the membrane center of the MF Column. Stand the MF Column for 1 min.
  - **Important step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
  - **Important:** Do not elute the DNA using less than suggested volume (10 µl). It will lower the final yield.
  - The average eluate volume is 10 µl from 12 µl elution buffer volume.
11. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute DNA.

## PCR Clean-Up Protocol: For purification of PCR products or reaction mixtures

Please Read Important Notes Before Starting Following Steps.

1. Transfer 10 ~ 100 µl of PCR product (excluding oil) to a microcentrifuge tube (not provided) and add 5 volumes of MF Buffer, mix well by vortexing.
  - For example, Add 250 µl of MF Buffer to 50 µl of PCR product.
  - The maximum volume of PCR product is 100 µl (excluding oil). Do not exceed this limit. If PCR product is more than 100 µl, separate it into multiple tubes.
2. Place a MF column into a Collection Tube.
3. Transfer the sample mixture to the MF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
4. Add 600 µl of Wash Buffer (ethanol added) to the MF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
  - Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.
5. Centrifuge again at full speed (~18,000 x g) for an additional 3 minutes to dry the column matrix.
  - **Important step!** The residual liquid should be removed thoroughly on this step.
6. Place the MF Column to a new microcentrifuge tube (not provided).
7. Add 10~12 µl of Elution Buffer or ddH<sub>2</sub>O to the membrane center of the MF Column. Stand the MF Column for 1 min.
  - **Important step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
  - **Important:** Do not elute the DNA using less than suggested volume (10 µl). It will lower the final yield.
  - The average eluate volume is 10 µl from 12 µl elution buffer volume.
8. Centrifuge at full speed (~18,000 x g) for 1 min to elute DNA.

## Troubleshooting

(For Gel Extraction)

Problems	Possible reasons	Solutions
The gel slice is hard to dissolve	Agarose gel of high percentage (> 2 %) is used	Add 5 volumes of FAGP Buffer to 1 volume of the gel slice.
	The size of the gel slice is too large	If the gel slice is more than 200 mg, separate it into multiple tubes.
Low or none recovery of DNA fragment	The column is loaded with too much agarose gel	The maximum volume of the gel slice is 200 mg per column.
	Elution of DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH <sub>2</sub> O is between 7.0- 8.5.
		Make sure that the elution solution has been completely absorbed by the membrane before centrifuge.
The size of DNA fragment is larger than 5 Kb	Preheat the elution solution to 60 °C before use.	
Eluted DNA contains non-specific DNA fragment	Contaminated scalpel	Using a new or clean scalpel.
	DNA fragment is denatured	Incubate eluted DNA at 95 °C for 2 min, then cool down slowly to reanneal denatured DNA.
Poor performance in the downstream applications	Salt residue remains in eluted DNA fragment	Wash the column twice with Wash Buffer.
	Ethanol residue remains in eluted DNA fragment	Do discard the flow-through after washing with Wash Buffer and centrifuge for an additional 3 min.

(For PCR Clean-Up)

Problems	Possible reasons	Solutions
Low or none recovery of DNA fragment	Apply more than 100 µl of PCR product	If PCR product is more than 100 µl, separate it into multiple tubes.
	Elution of DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH <sub>2</sub> O is between 7.0- 8.5.
		Make sure that the elution solution has been completely absorbed by the column membrane before centrifugation.
The size of DNA fragment is larger than 5 Kb	Preheat the elution solution to 60 °C before use.	
Poor performance in the downstream applications	Salt residue remains in eluted DNA	Wash the column twice with Wash Buffer.
	Ethanol residue remains in eluted DNA	Do discard the flow-through after washing with Wash Buffer and centrifuge for an additional 3 min.