

### FavorPrep<sup>TM</sup> Gel/PCR Purification Micro Kit

-For extraction of DNA fragments from agarose gel -For purification of PCR products or reaction mixtures (concentration and desalination of reaction mixtures) Cat. No.: FAGC1010 FAGC1013 FAGC1014 (For Research Use Only)

#### **Kit Contents:**

Cat. No:	FAGC1010	FAEGC1013	FAGC1014		
	(4 preps)	(50 preps)	(100 preps)		
MF Buffer Wash Buffer (Concentrate) <sup>a</sup> Elution Buffer MF Columns* Collection Tubes User Manual	1.5 ml × 2	30 ml	60 ml		
	1 ml	12.5 ml	20 ml		
	0.5 ml	5 ml	5 ml		
	4 pcs	10 × 5 pcs	10 × 10 pcs		
	4 pcs	50 pcs	100 pcs		
	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		

<sup>\*</sup>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

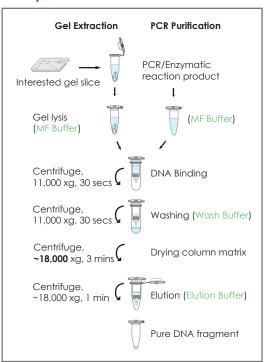
85%~95% for PCR clean up

Operation time: 10~20 mins Minimum Elution volume: 10 µl

### **Important Notes:**

- 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
- 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  $\mu$ l and the maximum amount of DNA fragments is 5  $\mu$ g.
- 5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000~18,000 xg.

### **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 mins and vortex the tube every 2~3 mins 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 xg for 30 secs, then discard the flow-through.
  - $\bullet\,$  If the sample mixture is more than 700  $\mu I$  , 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 xg for 30 secs, 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 xg) for an additional 3 mins 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 µl of Elution Buffer or ddH2O 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 µI). It will lower the final yield.
  - The average eluate volume is 10 µl from 12 µl elution buffer volume.
- 11. Centrifuge at full speed ( $\sim$  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.

- 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 MP Buffer to 50 µl of PCR product.
  - The maximum volume of PCR product is 100 µl (excluding oil). Do not excess 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 µl of Elution Buffer or ddH2O 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.
  - 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 ( $\sim$ 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 perfor- mance 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 perfor- mance 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.