FavorPrep™ GEL/ PCR Purification 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.: FAGCK 000
FAGCK 001-2
FAGCK 001-3

(For Research Use Only)

Kit Contents:

Cat. No:	FAGCK 000 (4 preps_sample)	FAGCK 001-2 (50 preps)	FAGCK 001-3 (200 preps)
FADF Buffer	3 ml	40 ml	160 ml
Wash Buffer (concentrate)[a]	1 ml	15 ml	45 ml
Elution Buffer	0.5 ml	5 ml	20 ml
FADF Column	4 pcs	50 pcs	200 pcs
Collection Tube	4 pcs	50 pcs	200 pcs
User Manual	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer[a]	4 ml	60 ml	180 ml

Specification:

Principle: spin column (silica matrix)

DNA Binding capacity of spin column: 20 µg

Sample size: up to 300 mg of agarose gel
up to 100 µl of reaction solution

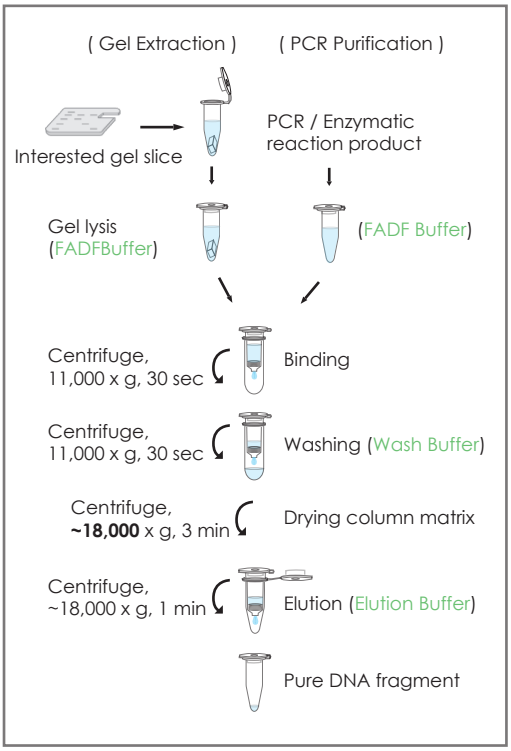
DNA size: 65 bp ~ 10 kbp

Recovery: 70% ~ 85% for Gel extraction
90% ~ 95% for PCR clean-up

Operation time: 10 ~ 20 min

Elution volume: 40 µl

Brief procedure:



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. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

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 300 mg of the gel slice into a microcentrifuge tube.(not provided).
- The maximum volume of the gel slice is 300 mg.

3. Add 500 µl of FADF Buffer to the sample and mix by vortexing.
- For > 2% agarose gels, add 1000 µl of FADF 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.
- After gel dissolved, make sure that the color of sample mixture is yellow. If the color is violet, add 10 µl of sodium acetate, 3M, pH 5.0. Mix well to make the color of sample mixture turned to yellow.

5. Cool down the sample mixture to room temperature. And place a FADF Column into a Collection Tube.

6. Transfer 800 µl of the sample mixture to the FADF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
- If the sample mixture is more than 800 µl, repeat this step for the rest of the sample mixture.

7. Add 750 µl of Wash Buffer (ethanol added) to the FADF 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 FADF Column to a new microcentrifuge tube (not provided).

10. Add 40 µl of Elution Buffer or ddH$_2$O to the membrane center of the FADF Column. Stand the FADF 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 (40 µl). It will lower the final yield.

11. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute the DNA.

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

Please Read Important Notes Before Starting Following Steps

1. **Transfer up to 100 µl of PCR product (excluding oil) to a microcentrifuge tube (not provided) and add 5 volumes of FADF Buffer, mix well by vortexing.**
   - For example, Add 250 µl of FADF 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 FADF column into a Collection Tube.**
3. **Transfer the sample mixture to the FADF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.**
4. **Add 750 µl of Wash Buffer (ethanol added) to the FADF 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 FADF Column to a new microcentrifuge tube (not provided).**
7. **Add 40 µl of Elution Buffer or ddH_2O to the membrane center of the FADF Column. Stand the FADF 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 (40 µl). It will lower the final yield.
8. **Centrifuge at full speed (~18,000 x g) for 1 min to elute the 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 1000 µl of FADF Buffer to 1 volume of the gel slice.
	The size of the gel slice is too large	If the gel slice is more than 300 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 300 mg per column.
	Elution of DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH_2O 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_2O 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.