FavorPrep™ GEL/PCR Purification Kit

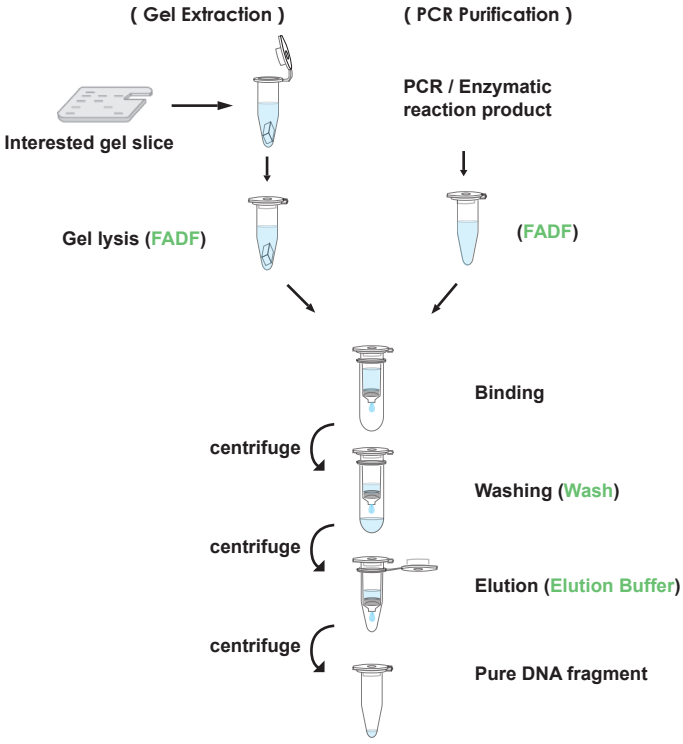
(Cat.: FAGCK 001-2, FAGCK 001-3)

(For Research Use Only)

Kit Contents:

	FAGCK001-2 (50 preps)	FAGCK001-3 (200 preps)
FADF Buffer	40 ml	160 ml
Wash Buffer*	15 ml	45 ml
Elution Buffer	5 ml	20 ml
FADF Column	50 pcs	200 pcs
2 ml Collection Tube	50 pcs	200 pcs

*Add 60 ml / 180 ml ethanol (96-100%) to Wash Buffer when first open.



Specification:

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

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

Operation time: 20 min
Elution volume: 40 µl

Important Notes:

1. Buffers provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
2. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

Gel Extraction Protocol:

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

1. Excise the 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 300mg.

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 10-15 minutes and vortex the tube every 2-3 min until the gel slice dissolved completely.
   - During incubation, interval vortex 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 FADF Column in a Collection Tube.

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

7. Add 750 µl of Wash Buffer (ethanol added) to the FADF Column. Centrifuge for 30 seconds then discard the flow-through.
   - Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.

8. Centrifuge again for an additional 3 minutes to dry the column.
   - Important step ! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

9. Place the FADF Column to a new microcentrifuge tube (not provided).

10. Add 40 µl of Elution Buffer or ddH2O to the membrane center of the FADF Column. Stand the FADF Column for 2 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 (40µl). It will lower the final yield.

11. Centrifuge for 2 min to elute the DNA.

12. Store the DNA at 4 °C or -20 °C.

PCR Clean-Up Protocol:

1. Transfer up to 100 µl of PCR product (excluding oil) and add 5 volumes of FADF Buffer to a microcentrifuge tube(not provided) then mix by vortexing.
   • 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 for 30 seconds then discard the flow-through.

4. Add 750 µl of Wash Buffer (ethanol added) to the FADF Column. Centrifuge 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 for an additional 3 minutes to dry the column.
   • Important step ! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

6. Place the FADF Column to a new microcentrifuge tube (not provided).

7. Add 40 µl of Elution Buffer or ddH2O to the membrane center of the FADF Column. Stand the FADF Column for 2 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 (40µl). It will lower the final yield.

8. Centrifuge for 2 min to elute the DNA.

9. Store the DNA at 4 °C or -20 °C.

Troubleshooting

The gel slice is hard to dissolve

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

(For Gel Purificaton) *The colulmn is loaded with too much agarose gel*
•The maximum volumn of the gel slice is 300 mg per column.

(For PCR Clean-Up) *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 ddH2O is between 7.0-8.5.
•Make sure the elution solution has been completely absorbed by the membrane before centrifugation.

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

(For Gel Purificaton) *Contaminated scalpel*
•Using a clean scalpel.

DNA fragment is denatured
•Incubate eluted DNA at 95°C for 2 minutes, 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
•Make sure you have discarded the flow-through after washing with Wash Buffer and centrifuged for an addition 3 minutes.