

Cat.: FAMGK000B, 4 Preps
 FAMGK001B, 50 Preps
 FAMGK001-1B, 200 Preps
 (For Research Use Only)

Kit Contents:

	FAMGK000B (4 preps)	FAMGK001B (50 preps)	FAMGK001-1B (200 preps)
MG Buffer	1.5ml x 2	65 ml	260 ml
Wash Buffer (concentrated)	1.0ml*	12.5 ml**	50 ml***
Elution Buffer	0.5ml	5 ml	5 ml
MG Column (blister packaging)	4 pcs	10 pcs x 5	10 pcs x 20
Collection Tube	4 pcs	50pcs	200 pcs

Add 4*, 50**, 200*** ml ethanol (96-100%) to Wash Buffer when first open.

Specification:

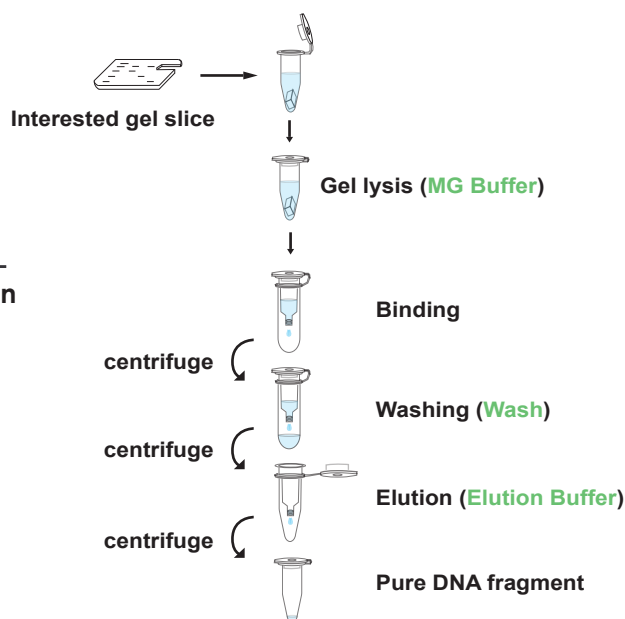
Sampling: agarose gel up to 200 mg

Recovery : 80-90%.

Binding capacity : 5 µg

Very small elution volume : 10-12 µl

Handling Time: 20 min for gel extraction.



Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffer.
2. Add required volume of ethanol (96~100%) to Wash Buffer as bottle indicated when first open.
3. For gel DNA extraction, excising the extra agarose gel to minimize the size of the gel. The maximum amount of gel slice is 200 mg; If the excised gel is more than 200 mg, separate it into multiple tubes.
4. All centrifuge steps are done at full speed (14,000 rpm) in a microcentrifuge.

Protocol:

Please Read Important Notes Before Starting The 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 1.5 ml microcentrifuge tube (not provided).
 - The maximum volume of the gel slice is 200 mg. If the excised gel is more than 200 mg, separate it into multiple tubes.
3. Add 3 volumes of MG Buffer to the sample and mix by vortexing.
 - For example, Add 600 µl of MG Buffer to 200 mg of gel.
 - For >2% agarose gels, Add 6 volume of MG Buffer.
4. Incubate at 55°C for 10 ~15 min and vortex the tube every 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. Add 1 gel volume of isopropanol to the sample and mix.
 - For example, if the gel is 200mg, add 200 µl isopropanol to the sample.
6. Transfer 600 µl of sample mixture to MG Column set. Centrifuge for 1 min then discard the flow-through.
7. Repeat step 6 for the rest sample mixture
8. Add 600 µl of Wash Buffer (ethanol added) to MG Column. Centrifuge for 30 sec then discard the flow-through.
 - Make sure that ethanol (96~100%) has been added into Wash Buffer when first open.

9. Centrifuge for an additional 1 min to dry MG column.

-Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

10. Place MG Column into a new 1.5 ml microcentrifuge tube (not provided).

11. Add 10-12 µl of Elution Buffer or ddH₂O to the membrane center of MG Column.

Stand MG Column for 2 min.

-The average eluate volume is 9 µl from 10 µl elution buffer volume.

-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 (12µl). It will lower the final yield. ↓

12. Centrifuge for 30 sec to elute DNA.

Troubleshooting

Problems	Possible reasons	Solutions
The gel slice is hard to dissolve	Agarose gel of high percentage (> 2 %) is used	Add 6 volumes of Gel Lysis Buffer 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 180 mg per column.
	Elution of DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH ₂ 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	Make sure you have discarded the flow-through after washing with Wash Buffer and centrifuged for an addition 3 minutes.