

## Kit Contents:

(For Research Use Only)

	FAVNK 000-1 (4 preps_sample)	FAVNK 001 (50 preps)	FAVNK 001-1 (100 preps)	FAVNK 001-2 (300 preps)
VNE Buffer	1.8 ml x 2	35 ml	70 ml	200 ml
Wash Buffer 1 * (concentrate)	0.9 ml x 2	22 ml	44 ml	132 ml
Wash Buffer 2 * (concentrate)	1.5 ml	20 ml	20 ml x 2	50 ml x 2
RNase-free Water	0.5 ml	6 ml	12 ml	20 ml
Carrier RNA	-----	0.4 mg	0.8 mg	2.2 mg
VNE Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
User Manual	1	1	1	1

\* Preparation of Wash Buffer W1 and W2 for first use:

Cat. No:	FAVNK000-1 (4 preps)	FAVNK001 (50 preps)	FAVNK001-1 (100 preps)	FAVNK 001-2 (300 preps)
ethanol volume for Wash Buffer 1	0.33 ml	8 ml	16 ml	48 ml
ethanol volume for Wash Buffer 2	6 ml	80 ml	80 ml	200 ml

## Specification:

Principle: spin column (silica membrane)

Sample: 150 µl cell-free fluid such as serum, plasma, body fluid and cell cultured supernatant

Fragment size: 100 bp ~30 kb

Recovery rate: 80 ~ 90 %

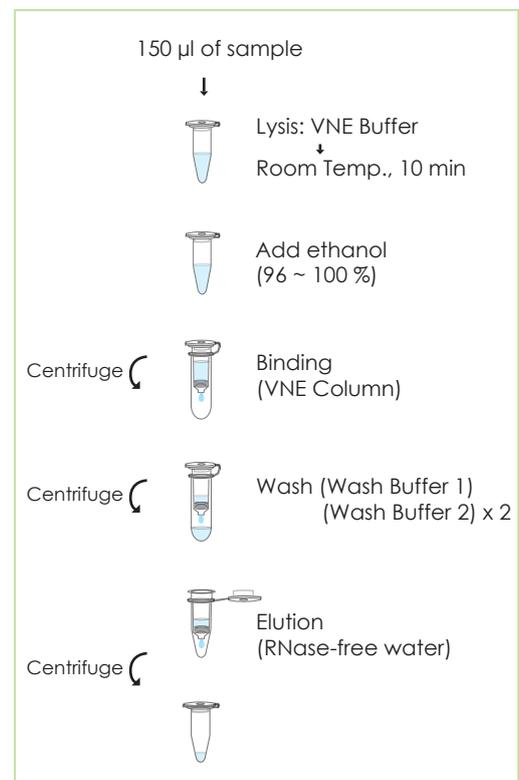
Binding capacity: 30 µg

Elution volume: 40 ~ 50 µl

Operation time: 20 min

## Important Notes:

1. Make sure everything is RNase-free when handling this system.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add 1 ml of VNE Buffer to the tube of lyophilized Carrier RNA, mix well by vortexing and transfer the mixture to the VNE Buffer when first open. Store the Carrier RNA added VNE Buffer at 4 °C.
4. Add required ethanol (96-100%) to Wash Buffer 1 and Wash Buffer 2 before use.
5. Preheat RNase-free water to 70°C for elution step. (step:10)



## General Protocol:

### Please Read Important Notes Before Starting Following Steps.

HINT: Preheat RNase-free water 70 °C for step 10 (elution step).

1. **Transfer 150 µl of sample (serum, plasma, body fluids or cell cultured supernatant ) into a microcentrifuge tube (not provided).**  
--If the sample volume is more than 150 µl, separate it into multiple tubes.
2. **Add 570 µl of VNE Buffer (Carrier RNA added) to the sample, mix well by vortexing, and incubate for 10 minutes at room temperature.**  
--Make sure that Carrier RNA has been added to the VNE Buffer when first use.
3. **Add 570 µl of ethanol (96~100%) to the sample mixture, mix well by plus-vortexing.**
4. **Combine a VNE column with a Collection Tube (provided). Transfer up to 700 µl of sample mixture (ethanol added) to the VNE Column, centrifuge at 8,000 x g for 1 min then discard the flow-through.**  
Combine the VNE Column with the used Collection Tube.
5. **Transfer the rest of sample mixture (ethanol added) to the VNE Column, centrifuge at 8,000 x g for 1 min.**  
Discard the flow-through and the Collection Tube. Combine the VNE Column with a new Collection Tube (provided).
6. **Add 500 µl of Wash Buffer 1 (ethanol added) to the VNE Column, centrifuge at 8,000 x g for 1 min then discard the flow-through.**  
Combine the VNE Column with the used Collection Tube.  
--Make sure that ethanol (96~100%) has been added into Wash Buffer 1 when first open.
7. **Add 750 µl of Wash Buffer 2 (ethanol added) to the VNE Column, centrifuge at 8,000 x g for 1 min then discard the flow-through.**  
Combine the VNE Column with the used Collection Tube.  
--Make sure that ethanol (96~100%) has been added into Wash Buffer 2 when first open.
8. **Repeat step 7. Add 750 µl of Wash Buffer 2 (ethanol added) to VNE Column, centrifuge at 8,000 x g for 1 min then discard the flow-through.** Combine the VNE Column with the used Collection Tube.
9. **Centrifuge at full speed (~18,000 X g) for an additional 3 min to dry the VNE column. Discard the flow-through and the Collection Tube.**  
--**Important step!** This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.
10. **Combine the VNE Column with a Elution Tube (provided). Add 50 µl of preheated RNase-free Water to the membrane center of the VNE Column. Stand VNE Column for 2 min.**  
--**Important step!** For effective elution, make sure that the RNase-free Water is dispensed onto the membrane center and is absorbed completely.
11. **Centrifuge for 2 min to elute the nucleic acid.**
12. **Store nucleic acid at -70 °C.**

## Troubleshooting

Problems	Possible reasons	Solutions
<b>Low or no yield of genomic DNA</b>		
	Incorrect preparation of Wash Buffer 1 or Wash Buffer 2	
	Wash Buffer W1 and Wash Buffer W2 is not mixed with ethanol before use	Make sure that the correct volumes of ethanol (96-100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first open. Repeat the extraction procedure with a new sample.
	The volume or the percentage of ethanol is not correct before adding into Wash Buffer W1 and Wash Buffer W2	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first use. Repeat the extraction procedure with a new sample.
	Elution of genomic DNA is not efficient	
	RNase-free water not completely absorbed by column membrane	After RNase-free water is added, stand the VNE Column for 2 min before centrifugation.
<b>Column is clogged</b>		
	Sample is too viscous	Reduce the sample volume.
<b>Degradation of elutated DNA</b>		
	Sample is old	Always use fresh or well-stored sample viral nucleic acid extraction.