

Kit Contents:

Cat. No:	APVNK 004 (4 preps, sample)	APVNK 050 (50 preps)	APVNK 100 (100 preps)	APVNK 200 (200 preps)
VNE Buffer	1.8 ml x 2	35 ml	70 ml	140 ml
Wash Buffer 1 * (concentrate)	0.9 ml x 2	22 ml	44 ml	88 ml
Wash Buffer 2 * (concentrate)	1.5 ml	20 ml	40 ml	40 ml x 2
RNase-free Water	0.5 ml	6 ml	6 ml x 2	20 ml
Carrier RNA	0.05 mg	0.4 mg	0.8 mg	1.6 mg
VNE Column	4 pcs	50 pcs	100 pcs	200 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	400 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	200 pcs
User Manual	1	1	1	1
* Preparation of Wash Buffer 1 and 2 for first use:				
ethanol volume for Wash Buffer 1	0.33 ml	8 ml	16 ml	32 ml
ethanol volume for Wash Buffer 2	6 ml	80 ml	160 ml	160 ml

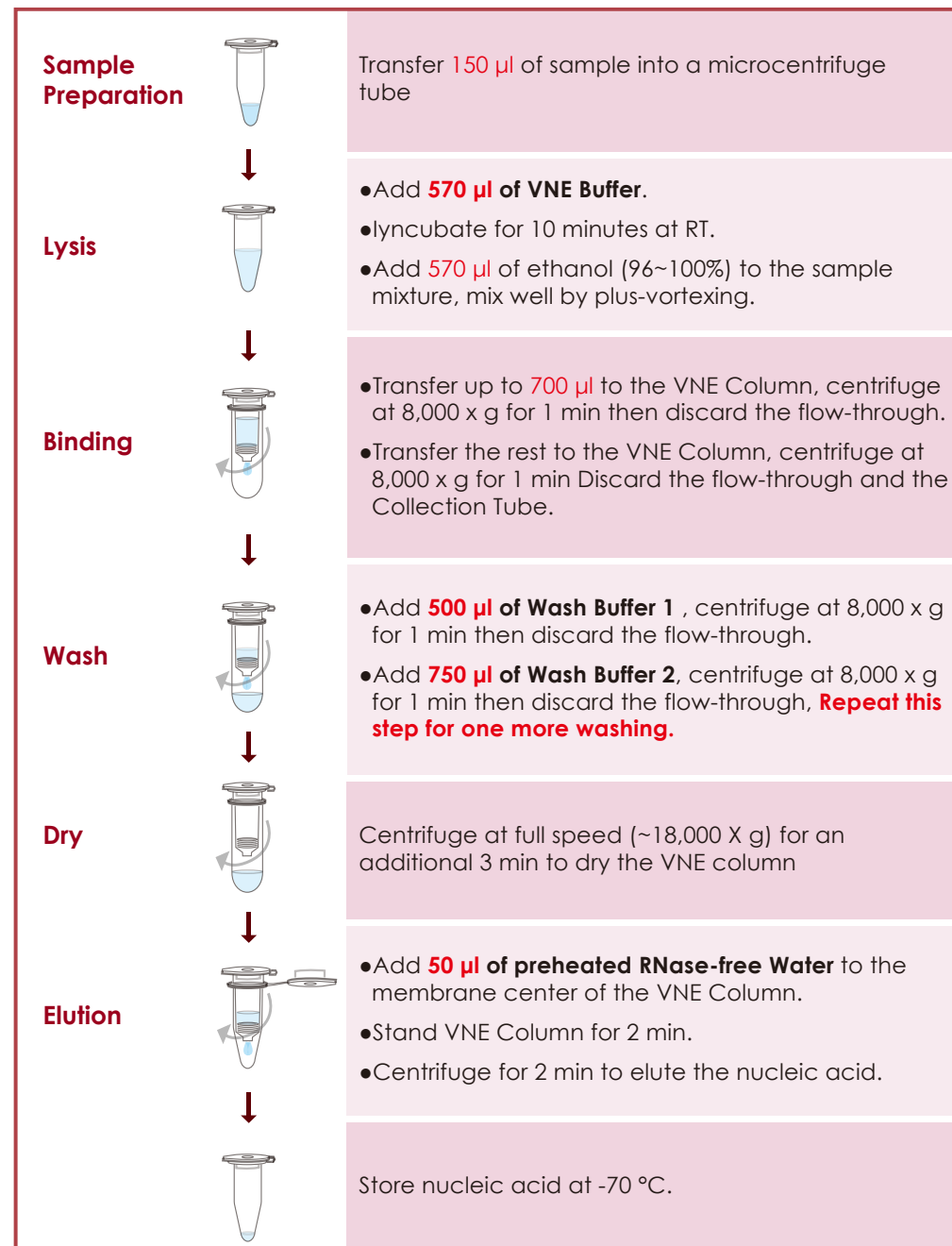
Specification:

Principle:	spin column (silica membrane)
Sample size:	150 μ l 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 μ g
Elution volume:	40 ~ 50 μ l
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.

Brief procedure:



General Protocol:

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	Transfer 150 µl of sample (serum, plasma, body fluids or cell cultured supernatant) into a microcentrifuge tube (not provided). HIT: •If the sample volume is more than 150 µl, separate it into multiple tubes. •If the sample is less than 150 µl, adjust the sample volume to 150 µl with PBS.
2 Lysis	•Add 570 µl of VNE Buffer (Carrier RNA added) to the sample, mix well by vortexing, and incubate for 10 minutes at room temperature. •Add 570 µl of ethanol (96~100%) to the sample mixture, mix well by plus-vortexing.
3 Binding	•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. •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).
4 Wash	•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. •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. •Repeat last step. 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.

5 Dry column	•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.
6 Elution	•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. •Centrifuge for 2 min to elute the nucleic acid. •Store nucleic acid at -70 °C.

