Viral Nucleic Acid Extraction Kit 2.0

SYG404-001/FYG404-100/FYG404-300

Store at RT Ver. T0428

Sample: 200 μl Sample (Serum, plasma, body fluids, cell culture supernatant), Swab

Yield: Up to 20 μg

Contents

	SYG404-001 (4 preps)	FYG404-100 (100 preps)	FYG404-300 (300 preps)
VN1 Buffer	1.5 ml	45 ml	125 ml
VN2 Buffer	220 μΙ	6 ml	16 ml
W1 Buffer	2 ml	45 ml	125 ml
W2 Buffer	300 μl x2	15 ml	25 ml x2
Elution Buffer	1 ml	10 ml	30 ml
VN Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
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Buffer Preparation

Add ethanol (96-100%) to the (VN2 Buffer/W2 Buffer) prior to first use.

	SYG404-001	FYG404-100	FYG404-300
	(4 preps)	(100 preps)	(300 preps)
VN2 Buffer	220 µl	6 ml	16 ml
Ethanol (96-100%)	1650 µl	45 ml	120 ml
W2 Buffer	300 μl x2	15 ml	25 ml x2
Ethanol (96-100%)	1.2 ml x2	60 ml	100 ml x2

Additional Requirements

- 1. PBS
- 2. DNase/RNase-free microcentrifuge tube

- 3. Ethanol (96-100%)
- 4. DNase I and reaction buffer (optional)
- 5. RNase (10 mg/ml) (optional)

Important Notes

- 1. Use sterile, RNase-free pipet tips and microcentrifuge tubes. Wear a lab coat and disposable gloves to prevent RNase contamination.
- 2. Make sure the starting sample amount is under the limit.
- 3. Add ethanol (96-100%) to (VN2 Buffer/W2 Buffer) prior to the initial use.

Purification Protocol:

Liquid samples

Step 1. Sample Preparation (liquid samples, 200 µl)

a. Transfer 200 µl sample (serum, plasma, body fluids or cell cultured supernatant) into a microcentrifuge tube (not provided).

(If the samples is less than 200 μ l, adjust the sample volume to 200 μ l with PBS.)

Step 2. Cell Lysis

- a. Add 400 µl **VN1 Buffer** to the sample, mix by vortexing.
- b. Incubate for 10 mins at room temperature.

Note: Preheat the Elution Buffer to 75 °C for the Elution step.

Optional Step (Prior to the Step2-c.): RNase treated

(If RNA-free genomic DNA is required, perform this optional step.)

i.Add 5 µl of RNase (10 mg/ml) to sample lysate and mix by vortexing.

ii.Incubate at room temperature for 5 mins.

c. Add 450 μ l **VN2 Buffer** (ethanol added) to the sample and shake vigorously.

Swab samples

Step 1. Sample Preparation

a. Soak the swab with 200 µl PBS.

Step 2. Cell Lysis

- a. Add 400 µl **VN1 Buffer** to the sample, mix by vortexing for 15 seconds.
- b. Incubate for 10 mins at room temperature.

Note: Preheat the Elution Buffer to 75 °C for the Elution step.

Optional Step (Prior to the Step2-c.): RNase treated

(If RNA-free genomic DNA is required, perform this optional step.)

- i. Add 5 µl of RNase (10 mg/ml) to sample lysate and mix by vortexing.
- ii. Incubate at room temperature for 5 mins.
- c. Add 450 μ l **VN2 Buffer** (ethanol added) to the sample and shake vigorously.

Step 3. Nucleic Acid Binding

- a. Place a **VN Column** in a 2 ml **Collection Tube**.
- b. Add 700 µl of lysate mixture from previous step to the **VN column**.
- c. Centrifuge at full speed (16,000 x g) for 1 min.
- d. Discard the flow-through and place the **VN Column** back in the **Collection Tube.**
- e. Apply the rest of sample mixture to the same column.
- f. Centrifuge at full speed (16,000 x g) for 1 min.
- g. Discard the flow-through and place the **VN Column** back in the **Collection Tube**.

Step 4-1. Wash

- a. Add 400 µl **W1 Buffer** add to the **VN Column**.
- b. Centrifuge at full speed (16,000 x g) for 30 seconds.
- c. Discard the flow-through and place the **VN Column** back in the **Collection Tube**.

Optional Step: DNase treated

(If DNA-free RNA is required, perform this optional step.)

- i. Add 150 µl **W2 Buffer** (ethanol added) into the **VN column**.
- ii.Centrifuge at full speed (16,000 x g) for 30 seconds.
- iii.Discard the flow-through and place the **VN Column** back in the **Collection Tube**.

iv.For each isolation reaction, premix 80 μl DNase I Incubation Buffer (not

provided) with 2 μ l DNase I (not provided) in a new sterile tube (Do not vortex).

v.Add 82 μ l of the DNase I solution to the center of the **VN Column** membrane and incubate at room temperature for 15 mins.

Step 4-2. Wash

- a. Add 600 µl **W2 Buffer (ethanol added)** to the **VN Column**.
- b. Centrifuge at full speed (16,000 x g) for 30 seconds.
- c. Discard the flow-through and place the **VN Column** back in the **Collection Tube**.

Step 5. Dry column

a. Centrifuge at full speed (16,000 x g) for 2 mins to dry the column matrix.

Step 6. Elution

- a. Place dried **VN Column** in a clean microcentrifuge tube (DNase-free/RNase-free, not provided).
- b. Apply 50-100 μ l of pre-Heated Elution Buffer to the center of the column matrix.

Note: For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.

- c. Stand for 2 mins until water absorbed by the matrix.
- d. Centrifuge at full speed (16,000 x g) for 2 mins to elute purified nucleic acid.

Step 7. Store nucleic acid

a. Store nucleic acid at -80 °C.

Product Use Limitation & Warranty

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