Viral RNA/ Viral Nucleic Acid Mini Kit DE-001, DR-002 , DR-003

- For isolation of viral RNA/ viral nucleic acid from cell-free fluid such as, serum, plasma, body fluid and cell culture supernatant, and from transport mediumof swabs

New protocol

Kit Contents:

Cat. No:	**DR-001-S** (4 preps_sample)	**DR-001** (50 preps)
VNE Buffer ■	1.8 ml x 2	35 ml
Carrier RNA ■	0.04 mg	0.4 mg
Wash Buffer 1 * □ (concentrate)	0.48 ml x 2	12 ml
Wash Buffer 2 □ (concentrate)	1.5 ml	20 ml
RNase-free Water	0.5 ml	6 ml
VNE Column	4 pcs	50 pcs
Collection Tube	8 pcs	100 pcs
Elution Tube	4 pcs	50 pcs
User Manual	1	1
■ □ , see ***Working Buffer Preparation.***		

Cat. No:	**DR-002 (100 preps)**	**DR-003 (300 preps)**
VNE Buffer ■	70 ml	200 ml
Carrier RNA ■	0.8 mg	2.2 mg
Wash Buffer 1* □ (concentrate)	24 ml	72 ml
Wash Buffer 2 □ (concentrate)	20 ml x 2	50 ml x 2
RNase-free Water	12 ml	20 ml
VNE Column	100 pcs	300 pcs
Collection Tube	200 pcs	600 pcs
Elution Tube	100 pcs	300 pcs
User Manual	1	1
■ □ , see ***Working Buffer Preparation.***		

**NEW FORMULATION (60% Ethanol added)*

Storage:

1. Kit components except Carrier RNA should be stored at room temperature (15 - 25 °C).
2. Carrier RNA should be stored at -20 °C upon receipt.
3. VNE Buffer should be stored at 4 °C after adding Carrier RNA.

Quality Control:

The quality of our Viral RNA/ Viral Nucleic Acid Mini Kit is tested on a lot-to-lot basis according to ISO quality management system.

Product description:

The Viral RNA/ Viral Nucleic Acid Mini Kit is an excellent tool for extraction of high pure viral nucleic acid from viral cellfree specimen such as, serum, plasma, body fluid and cell cultured supernatant, and from transport medium of swabs.
The extraction method is based on the silica membrane/ chaotropic salt technology, and the procedure involves lysis of virus, optimization of binding condition being able to make the viral nucleic acid efficiently to silica membrane, washing silica membranes to remove contaminations including salts, metabolites, nucleases and other components of body fluid, finally elution of the viral nucleic acid from the silica membrane.

The provided Carrier RNA collaborate with the unique lysis buffer will elevate the efficiency of binding nucleic acid to the silica membrane and become a substrate to be cleaved by nuclease containing in the sample mixture, thus elevate the integrity and the recovery of viral nucleic acid. Compare with other harmful and time-consuming method, such as phenol/ chloroform extraction and ethanol precipitation, the Viral RNA/ Viral Nucleic AcidMini Kit makes extraction of high-purity viral nucleic acid reliable, and that shortens the handling time less than **20 minutes for one preparation**.

Product Specification:

Format/ Principle: spin column/ silica membrane/ chaotropic salt
Sample size: 140 µl cell-free fluid such as serum, plasma, body fluid and cell cultured supernatant
Operation time: < 20 min
Recovery rate: 80 ~ 90 %
Length of recovery nucleic acid: > 200 bp
Column Binding capacity: 60 µg RNA /column
Elution volume: 40 ~ 50 µl
Column applicability: centrifugation and vacuum

Materials and equipments provided by the user

For All Protocol:
- Pipets, pipet tips and centrifuge tubes (1.5 ml, 2.0 ml), sterile
- 96 ~100 % ethanol (for preparation of Wash Buffer 1&2.

For centrifuge processing:
- A micro-centrifugator is capable of ~18,000 X g, with a rotor for 1.5 or 2.0 ml micro-centrifuge tube.

For vacuum processing:
- A micro-centrifugator is capable of ~18,000 X g, with a rotor for 1.5~2.0 ml micro-centrifuge tube.
- A vacuum manifold contains adaptors for VNE columns, and the vacuum be capable to -6 inches Hg.

Working Buffer Preparations:

■ **Preparation of VNE-Carrier RNA Buffer**
Add 1 ml of VNE Buffer to the tube containing lyophilized Carrier RNA. Mix well by vortexing and transfer the mixture to the VNE Buffer when first open. Store the VNE- Carrier RNA Buffer at 4 °C.

□. **Preparation of Wash Buffer 1 and Wash Buffer 2**
Add required ethanol (96~100%) as the table below indicated.

Store the Wash Buffer 1 & 2 (ethanol added) at 15~25 °C.

Cat. No:	DR-001-S (4 preps)	DR-001 (50 preps)
ethanol volume for Wash Buffer 1	0.72 ml, each	18 ml
ethanol volume for Wash Buffer 2	6 ml	80 ml

Cat. No:	DR-002 (100 preps)	DR-003 (300 preps)
ethanol volume for Wash Buffer 1	36 ml	108 ml
ethanol volume for Wash Buffer 2	80 ml	200 ml

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Safety Information:

CAUTION: VNE Buffers and Wash Buffer 1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions directly to the preparation waste.**

Kit Component: **VNE Buffer**

Hazard contents
Guanidinium thiocyanate
CAS-No. 593-84-0
EC-No. 209-812-1

GHS symbol  Warning

Hazard statement(s)

H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing / eye protection/ face protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Kit Component: **Wash Buffer 1**

Hazard contents
Guanidine hydrochloride, 20~50%, CAS-No. 50-01-1

GHS symbol  Warning

Hazard statement(s)

H302	Harmful if swallowed.
H319	Causes serious eye irritation.

Precautionary statement(s)

P264	Wash … thoroughly after handling.
P280	Wear protective gloves/ protective clothing/ eye protection/ fac protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.

Important notes:

1. Notes for sample preparation:

- Make sure everything is RNase-free when handling this system.
- Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
- Do not thaw the frozen plasma or serum samples more than once.
- Centrifuge the plasma or serum samples at 6,000 x g for 3 minutes If precipitates are visible. Then transfer the cleared supernatant to a new vial and processed immediately.

2. Notes for Buffers:

- Add Carrier RNA to the VNE Buffer when first open. Store the VNE-Carrier RNA Buffer at 4 °C. see ***Working Buffer Preparation.***
- Add required ethanol (96-100%) to Wash Buffer 1 and Wash Buffer 2 before use. see ***Working Buffer Preparation.***
- For handling the buffers safely please read ***safety Information*** before starting the procedure.

3. Notes for centrifuging and vacuum:

- Ensure that centrifugation speed is according to instruction of individual step.
- When using of vacuum to proceed "RNA/ nucleic acid to column membrane" and " Wash column membrane", ensure that the tip of the column is fit into the shape of manifold adaptor and vacuum pressure being capable to reach to -6 inches Hg.
- Units and values at same pressure (1 atm)

unit	value
atmosphere (atm)	1.000
millimeter of mercury (mmHg)	760.000
inches of mercury (inHg)	29.290
pascal (Pa)	101,325.000
kilopascal (KPa)	101.325
torr (torr)	760.000
pound per square inch (psi, 1bs/in²)	14.700

Brief procedure:

- Sample type

(1) serum, plasma, body fluid or cell cultured supernatant

140 µl of sample

(2) swabs samples in transport medium

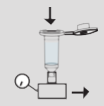
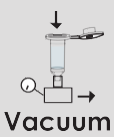
Transfer 140 µl of medium

•Sample lysis (***VNE Buffer***)

Add 560 µl of VNE Buffer
↓
Plus-Vortex, 5 sec
↓
Incubate at R.T, 10 min

• optimization of binding condition (adding ***ethanol***)

Add 560 µl of ethanol (96 ~ 100 %)
↓
Plus-Vortex, 5 sec

	Centrifuge	Vacuum
• Bind viral nucleic acid to column membrane (***VNE Column)***	Centrifuge 8,000 x g, 30 sec	 Vacuum -6 inches Hg
• Wash column membrane ***(Wash Buffer 1)*** ***(Wash Buffer 2) x 2***	 Centrifuge 8,000 x g, 30 sec	 Vacuum -6 inches Hg
• Dry membrane	 Centrifuge ~18,000 x g, 1 min	
• Elute viral nucleic acid (***RNase-free water***)	 Centrifuge ~18,000 x g, 30 sec ↓  • Pure viral nucleic acid	

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Centrifuge Protocol:

Please Read Important Notes Before Starting Following Steps.

• Sample type

A. Cell-free fluid such as Serum, plasma, body fluids and cell cultured supernatant

1-A1. Briefly spin the tube to descend the drops attached on the tube wall. **Note!** Centrifuge the sample at 7,000 x g for 3 minutes If the precipitates are visible.

1-A2. Transfer 140 µl of the fluid sample (cleared supernatant) to a microcentrifuge tube (not provided).

B. Medium of transport swabs

1-B1. Briefly vortex the swabs transport tube then briefly spin the tube to descend the drops attached on the tube wall.

1-B2. Transfer 140 µl of the medium to a microcentrifuge tube (not provided).

• Sample lysis

2. Add 560 µl of VNE - Carrier RNA Buffer (Carrier RNA added, **see Working Buffer Preparation**). Mix well by vortexing and incubate for 10 minutes at room temperature.

• Optimization of binding condition

3. Add 560 µl of ethanol (96~100 %) to the sample mixture and mix well by plus-vortexing.

• Bind viral DNA/ RNA to column membrane (centrifuge)

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 and 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).

• Wash column membrane (centrifugation)

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 650 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.

• Dry membrane

9. Centrifuge at full speed (~18,000 X g) for 1 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.

• Elute Viral RNA

10. Combine the VNE Column with an Elution Tube (provided). Add 40 ~ 60 µl of RNase-free Water to the membrane center of the VNE Column. Stand VNE Column for 1 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 at full speed (~18,000 X g) for 1 min to elute the viral DNA/ RNA. Store the viral DNA/ RNA at -70 °C.

Vacuum Protocol:

Please Read Important Notes Before Starting Following Steps.

• Sample type

A. Cell-free fluid such as Serum, plasma, body fluids and cell cultured supernatant

1-A1. Briefly spin the tube to descend the drops attached on the tube wall. **Note!** Centrifuge the sample at 7,000 x g for 3 minutes If the precipitates are visible.

1-A2. Transfer 140 µl of the fluid sample (cleared supernatant) to a microcentrifuge tube (not provided).

B. Medium of transport swabs

1-B1. Briefly vortex the swabs transport tube then briefly spin the tube to descend the drops attached on the tube wall.

1-B2. Transfer 140 µl of the medium to a microcentrifuge tube (not provided).

• Sample lysis

2. Add 560 µl of VNE - Carrier RNA Buffer (Carrier RNA added, **see Working Buffer Preparation**). Mix well by vortexing and incubate for 10 minutes at room temperature.

• Optimization of binding condition

3. Add 560 µl of ethanol (96~100 %) to the sample mixture and mix well by plus-vortexing.

• Bind viral DNA/ RNA to column membrane (vacuum)

4. Combine the tip of a VNE Column with the adaptor of the vacuum manifold. Retain the Collection Tube for be used on step 9. Transfer up to 700 µl of sample mixture (ethanol added) to the VNE Column and apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
5. Transfer the rest of the sample mixture (ethanol added) to the VNE Column and apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

• Wash column membrane (vacuum)

6. Add 500 µl of Wash Buffer 1 (ethanol added) to the VNE Column. Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
7. Add 650 µl of Wash Buffer 2 (ethanol added) to the VNE Column Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
8. Repeat step 7.

• Dry membrane

9. Remove the VNE Column from manifold and return the VNE Column back to the Collection Tube. Centrifuge at full speed (~18,000 X g) for 1 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.

• Elute Viral RNA

10. Combine the VNE Column with an Elution Tube (provided). Add 40 ~ 60 µl of RNase-free Water to the membrane center of the VNE Column. Stand VNE Column for 1 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 at full speed (~18,000 X g) for 1 min to elute the viral DNA/ RNA. Store the viral DNA/ RNA at -70 °C.

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Troubleshooting

- **Low yield**
  - ■ Carrier RNA not add to VNE Buffer or VNE-Carrier Buffer not store well
    - □ Add 1 ml of VNE Buffer to Carrier RNA. Mix well and transfer mixture to the VNE Buffer and store the VNE-Carrier RNA Buffer at 4 °C.
  - ■ Sample not store well or repeated freeze–thaw
    - □ Store samples at – 80 °C for long-term storage. Frozen samples do not be thawed more than once.
  - ■ RNA Degradation
    - □ Harvested samples not immediately stabilized.
  - ■ Insufficient mixing with VNE-Carrier RNA Buffer
    - □ Mix the sample mixture by plus-vortexing
  - ■ Insufficient lysis of protein
    - □ Incubate the sample mixture at room temperature for 10 minutes after adding VNE-Carrier RNA Buffer.
  - ■ Improper RNA binding condition
    - □ No ethanol added to the lysate (step 3) or incorrect percentage of ethanol be used.
  - ■ Incorrect RNA elution
    - □ Ensure that RNase free water was added at the center of the VNE column membrane and absorbed by the membrane.
  - ■ Incorrect preparation of Wash Buffer 1&2
    - □ Ensure that the correct volume of ethanol (96~100 %) was added to Wash Buffer 1&2 when first use.

- **Eluted RNA does not perform well**
  - ■ Residual ethanol contamination
    - □ Ensure that VNE Column has done centrifugation for an additional 1 min at speed ~18,000 x g (step 9) after washing step.

OTHER PRODUCTS FISHER MOLECULAR BIOLOGY:

Nucleic Acid Extraction - spin column (silica membrane)
- Viral DNA/ RNA Kit
- Viral RNA/ DNA Vacuum Kit

RNA Extraction - spin column (silica membrane)
- Blood/Cultured Cell Total RNA ***Mini/ Maxi*** Kit
- Soil RNA Isolation Mini Kit
- Tissue Total RNA ***Mini/ Maxi*** Kit
- Plant Total RNA ***Mini/ Maxi*** Kit
- After Tri-Reagent RNA Clean-Up Kit

96-Well high throughput DNA/ RNA extraction (silica membrane)
- 96-well Gel/ PCR purification kit
- 96-well PCR Clean-Up Kit
- 96-Well Total RNA Kit
- 96 well Viral DNA/RNA extraction kit
- 96-Well Genomic DNA Extraction Kit
- 96-Well Plasmid Kit

Plasmid Extraction
- ***Mini/ Midi/ Maxi*** plasmid kit - spin column (silica membrane)
- ***Midi/ Maxi*** plasmid kit - gravity flow column (anion -exchange resin)
- Endotoxin Free ***Midi/ Maxi*** plasmid kit - gravity flow column (anion-exchange resin)

DNA Clean-Up - spin column (silica membrane)
- PCR Clean-UP Kit/ • GEL Purification Kit/ • GEL/PCR Purification Kit
- ***MicroElute*** GEL/PCR Purification Kit

DNA Extraction - spin column (silica membrane)
- Blood / Cultured Cell Genomic DNA Extraction ***Mini /Midi/ Maxi*** Kit
- Plant Genomic DNA Extraction ***Mini/ Maxi*** Kit
- Food DNA Extraction Kit
- Milk Bacterial DNA Extraction Kit
- Tissue Genomic DNA Extraction Mini Kit
- FFPE Tissue DNA Extraction ***MicroElute*** Kit
- Fungi/ Yeast Genomic DNA Extraction Mini Kit
- Soil DNA Isolation Mini Kit
- Stool DNA Isolation Mini Kit

Extraction Reagent
- Tri-RNA Reagent - (Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction)

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