

Viral RNA Purification Kit (BW-VR6531)

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Kit Contents

Catalog#	BW-VR6531-00	BW-VR6531-01	BW-VR6531-02
Preps	10	50	250
Buffer HLY	7 mL	35 mL	175 mL
L Solution	26 µL	130 µL	650 µL
Proteinase K	320 µL	1600 µL	8 mL
RNA Wash Buffer*	3 mL	15 mL	75 mL
Buffer MKB	7 mL	35 mL	175 mL
DEPC-Treated ddH ₂ O	1 mL	3 mL	15 mL
MV RNA Micro Columns	10	50	250
gDNA removal Micro Columns	10	50	250
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*Add 12 mL (BW-VR6531-00), 60 mL (BW-VR6531-01) or 300 mL (BW-VR6531-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

Purpose: This procedure describes the extraction of viral RNA including COVID-19 and other RNA virus infected specimen for real-time RT-PCR detection of COVID-19 in respiratory specimens and sera.

Protocol use limitations: The RNA extraction protocol described here has not been validated for platforms or chemistries using clinical samples.

Acceptable Specimens

- Respiratory specimens including: nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, bronchoalveolar lavage, tracheal aspirates, and sputum. Swab specimens should be collected only on swabs with a synthetic tip with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.
- Serum, plasma, or other body fluid.

Storage and Stability

The guaranteed shelf life is 12 months from the date of production. L Solution and Proteinase K store at -20°C. All other components can be stored at room temperature (15-25°C).

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each step.

Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified class III biological safety cabinet following biosafety level 3 or higher guidelines.

Important

- ☀ Calculate and aliquot amount of Buffer HLY to be used in a clean tube and add 10 µL β-mercaptoethanol (β-Me) or 40 µL DTT (1 M) to 1 mL Buffer HLY. Add 4 µL of L Solution per 1 mL of Buffer HLY/β-Me or Buffer HLY/DTT. Buffer HLY contains β-Me or DTT can be stored at room temperature up to 1 month.
- ☀ Add 12 mL (BW-VR6531-00), 60 mL (BW-VR6531-01) or 300 mL (BW-VR6531-02) 96-100% ethanol to each RNA Wash Buffer bottle before use. The final ethanol is 80% (v/v).

Materials not Supplied

- ☀ Tabletop microcentrifuge.
- ☀ β-mercaptoethanol or DTT.
- ☀ 96-100% ethanol.

Safety Information

Buffer HLY and Buffer MKB contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation

waste, wear gloves and protective eyewear when handling.

Protocol (For extracting viral RNA from infected specimen)

1. Add **30 µL Proteinase K** to 300 µL sample. Mix well. Add **300 µL Buffer HLY-L solution** by vortexing for 5 seconds.

Note: The sample range 200-400 µL.

2. Incubate the sample at 30°C for 10 minute.
3. Transfer the lysate to a **gDNA removal Micro Column** and spin at 10,000 rpm for 30 seconds.
The RNA is in the flow through.
4. Add equal volume **ethanol** (96–100%) to the flow through, and mix by pulse-vortexing for 20 s.
After mixing, briefly centrifuge the tube to remove drops from inside the lid.
5. Transfer the lysate to a **MV RNA Micro Column** and spin at 12,000 rpm for 1 minute. Discard the 2 mL Collection Tube with the flow-through and put the column back to a new collection tube.
6. Add **600 µL Buffer MKB** to the column and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
7. Add **600 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 seconds.
Discard the flow-through.
8. Repeat step 7.
9. Centrifuge the empty column, with the lid open, at 12,000 rpm for 2 minutes.

Note: It is critical to remove residual ethanol for optimal elution

10. Place the column to a **1.5 mL RNase-free Microfuge Tube**, add **35-50 µL DEPC-Treated ddH₂O** to the column and centrifuge at 12,000 rpm for 1 minute. Store the purified RNA at -20°C.
11. Optional: Add the eluent back to the column for a second elution.

Note: The first elution normally yield 60-70% of the RNA while the second elution yield another 20-30% of the RNA bound to the column.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.



Contact Us: [400-115-2855](tel:400-115-2855)

www.beiwobiomedical.com

Customer Support:

market@beiwobiomedical.com

Technical Support:

tech@beiwobiomedical.com