NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

SHENTEK Biofargo

www.biofargo.com
804-529-2296
contact@biofargo.com

SHENTEK

Virus DNA & RNA Extraction Kit

User Guide

Version: A/1
For Research Use Only
Product No.: 1506730
Reagents for 50 Extractions

Biofargo, Inc.

(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK®Virus DNA & RNA Extraction Kit is suitable for extraction of viral DNA and RNA from up to 10^7 cells, and also for nucleic acid extraction of harvested virus bulk (liquid). The kit is compatible with either manual sample preparation or automated extraction with SHENTEK® rHCDpurify system.

Depending on subcellular location of viral replication, the extraction methods for cells can be grouped into two categories:

Method 1: For viruses replicated in host nucleus including retroviruses, such as most DNA viruses (rcAAV/rAAV) as well as replication competent lentivirus (RCL) and relication competent retrovirus (RCR), additional nuclease digestion after cell lysis can reduce the risk of false negative. Method 1 is also recommended for detection of samples contaminated with unknown viruses.

Method 2: For viruses replicated in host cytoplasm, for example most RNA viruses, cell lysis followed by centrifugation and extraction of the supernatant is sufficient for detection purposes.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, mask and gloves.

Table 1. Kit component and storage

Reagent	Part No.	Quantity	Storage
Wash buffer A	NND015	15 mL × 1 bottle	room temperature
Binding solution	NND017	10 mL × 1 bottle	
Proteinase K Buffer	NND025	5 mL × 1 bottle	
Pretreatment buffer	NND002	1.25 mL × 2 tubes	2-8℃
Magnetic particles	NND030	750 μL × 2 tubes	
5M NaCl	NND040	500 μL × 1 tube	
Precipitation solution I	NND003	25 μL × 1 tube	-20℃
RNase-Free H_2O	NND008	1.2 mL × 6 bottles	
Proteinase K	NND023	500 μL × 5 tubes	

The kit components can be stored at the appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Required materials not included in the kit

- Anhydrous Ethanol (AR)
- 100% Isopropanol(AR)
- Nonstick, low retention tips: 1000μL, 100μL and 10μL
- Nonstick, RNase-free microfuge tubes: 1.5mL, 2.0mL and 50mL
- RNase inhibitor
- DEPC water (for the RNA virus)
- Nuclease and nuclease buffer (or use 100 mM $MgCl_2$)

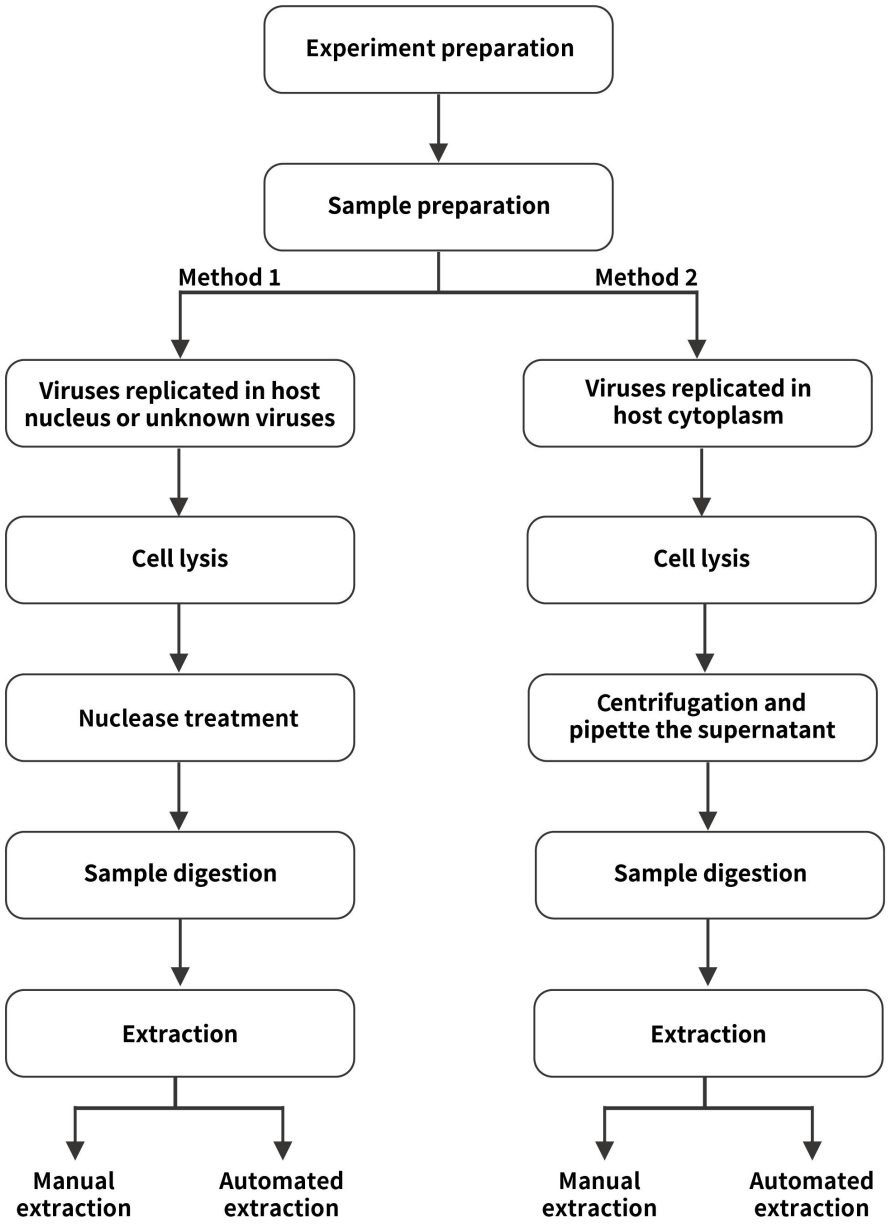
■ Related equipment

- Benchtop microcentrifuge
- Magnetic stand or rHCDpurify system
- High-speed refrigerated centrifuge
- Vortex mixer
- Dry bath incubator
- Micropipettes: 1000μL, 100μL, and 10μL

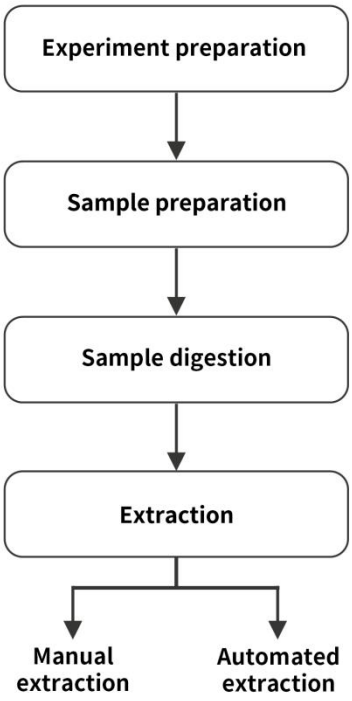
- Biosafety cabinet
- Microplate and microtube shaker

2. Methods

■ Viral Nucleic Acid Extraction Procedure for Cell Samples



■Viral Nucleic Acid Extraction Procedure for Harvested Virus Bulk (Liquid)



■ Experiment preparation

Before first use of the kit:

- Add 20 mL of Anhydrous Ethanol to Wash buffer A.
- Prepare 70% ethanol buffer using DEPC water, and label as Wash buffer B.
- Store Wash buffer at room temperature (RT) properly to prevent evaporation by expiration date.

Before each use of the kit:

- Prepare 100% Isopropanol.
- Set the dry bath (or water bath) temperature to 37°C and 55°C, and additional 70°C dry bath are needed for manual extraction method.

  Note: If the Protease K Buffer or Binding Solution is cloudy or contains precipitates, heat at 37°C until it clears.
- Incubate the magnetic particles (NND030) suspension at RT for 10 min, and vortex to mix well before use.

Note: To guarantee the binding ability of magnetic particles, aliquot the magnetic particles before the first use to avoid frequent temperature shift.

- Precipitation solution I dilution: According to the volume of binding buffer needed for your experiment, dilute precipitation solution I (NND003) with RNase-Free H_2O (NND008) at a ratio of 1:99.

  Note: Prepare the total volume of binding buffer appropriately more than necessary for experiment to compensate for pipetting loss (the necessary volume is calculated based on the volume for one sample and total sample amount).

■ Sample pretreatment

Note: To guarantee the accuracy of the test, duplicates are recommended for each sample.

- Negative control samples (NCS)

  NCS is prepared in the same procedure as test samples during extraction. NCS will serve as a blank to evaluate whether there is cross contamination or environmental contamination during handling.

- For RNA viruses and unknown samples, RNase inhibitor (final concentration of 1U/μL) should be added to the test samples, vortex for 10 seconds and quickly centrifuge for 3-5 seconds.

For test samples (Please choose the appropriate procedure accordingly)

- **Cell culture samples (≤ 10^7 cell)**

  1) Sample pretreatment

  Add Pretreatment buffer (NND002) to the sample at the ratio of 1:10, and vortex to mix well. Incubate at RT for 5 min.

Method 1 (For viruses replicated in host nucleus or unknown viruses)

Add 100 U of nuclease and nuclease buffer (or use 100 mM $MgCl_2$ instead of buffer, the final concentration of $MgCl_2$ is about 2 mM), vortex for 10 seconds and quickly centrifuge for 3-5 seconds, and incubate sample at 37℃ for 30 min.

Method 2 (For viruses replicated in host cytoplasm)

After pretreatment buffer incubation, centrifuge at 450×g for 30 min, carefully transfer the supernatant to a new microfuge tube, and discard the pellets

■ Samples digestion

1. Add 100 μL of Protease K Buffer and 50 μL of Protease K to each sample tube, vortex to mix well, then spin 3-5 seconds in a microcentrifuge. Incubate all samples including the control samples at 55°C for 30 min

2. Add 10 μL 5M NaCl and 10 μL of diluted Precipitation solution I (please see Before each use of the kit - Precipitation solution I dilution) to each sample tube, vortex to mix well, then spin 3-5 seconds in a microcentrifuge.

Note: After sample digestion, the downstream nucleic acid extraction experiment should be performed as soon as possible.

■ Nucleic acid extraction

Manual procedure

Binding of DNA&RNA

1. Incubate the magnetic particles at RT for 10 min, and vortex to mix well before use.

2. Take out the sample from the dry bath and spin for 30 seconds, then add 200 μL Binding buffer, vortex to mix well and spin for 10 seconds.

3. Add 200-400 μL of Isopropanol and 30 μL of magnetic particles suspension. Vortex the tubes vertically at medium speed for 1 min to bind the nucleic acids, and spin for 3-5 seconds, repeat this step for 4-5 times, afterwards place the tubes on the magnetic stand.

4. Wait until the solution is clear and the beads are completely separated, discard the supernatant without disturbing the beads.

Note: The time to reach complete clear solution and beads separation is about

3-5 minutes.

The volume of isopropanol is approximately 1/3 to 1/2 of the total liquid volume.

When transferring the supernatant, avoid to remove the magnetic particles with the supernatant. During extraction, when the sample tubes are placed into the magnetic stand, always orient the Magnetic particle pellet toward the magnet.

Washing DNA&RNA

1. Take each tube from the magnetic stand, and add 700μL of Wash buffer A. Vortex to mix well for 10 seconds and spin for 10 seconds in a microcentrifuge, then place the tubes on the magnetic stand. Discard the supernatant without disturbing the magnetic particles.

2. Continue to take each tube from the magnetic stand, and add 700μL of Wash buffer B. Vortex for 40 seconds and spin for 10 seconds, then place the tubes on the magnetic stand. Discard the supernatant without disturbing the magnetic particles.

3. To remove the supernatant completely, spin in a microcentrifuge for 10 seconds and place each tube on the magnetic stand again. Wait until the beads are completely separated, carefully use a 10 μL volume pipette to remove the residual liquid.

4. With the cap open, air-dry the magnetic particles pellet at RT for 30 seconds to 3 min to remove any residual ethanol.

Note: When transferring the supernatant, avoid to remove the magnetic particles with the supernatant.

The drying time depends on the specific environmental conditions. It could be shorter in higher temperature or lower humidity condition, while slightly longer in lower temperature or higher humidity condition.

Eluting DNA&RNA

1. Add 50-100 μL RNase-Free H_2O (preheated at 70℃) to each sample, vortex for 5 seconds and incubate at 70℃ for 7 min. Vortex 2 - 3 times during incubation

to ensure complete resuspension of magnetic particles.

Note: Vortex the mixture of magnetic particles and eluent, then swing to the bottom of the tube.

If the mixture splashed to the cap, centrifuge the tube and mix well by vortexing.

2. After incubation, quickly spin the tube for 1 min, then place the tubes on the magnetic stand. Wait until the beads are completely separate, carefully transfer the eluate to a new microfuge tube.

3. Quickly spin the tube for 10 seconds, and then place it on the magnetic stand again. Carefully transfer the eluate to a new microcentrifuge tube and label the corresponding sample name.

Note: Transfer the eluate completely and avoid leaving any residuals behind.

Automated procedure with rHCDpurify system

1. Plate preparation

During digestion, add the corresponding solution according to Table 2:

Table 2. 96-deep well plate layout

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1											
S2											
S3											
S4											
S5											
S6											
S7											
S8						NCS					

- Column 1 or 7: binding buffer 200 μL/well, isopropanol 200-400 μL/well and all samples after digestion
- Column 2 or 8: Washing buffer A 700 μL/well

- Column 3 or 9: Washing buffer B 700 μL/well
- Column 4 or 10: Magnetic particles 30 μL/well
- Column 5 or 11: RNase-Free H_2O 50-100 μL/well

Note:

Incubate the magnetic particles suspension at RT for 10 min, and vortex to mix well before use.

To guarantee the binding ability of magnetic particles, aliquot the magnetic particles suspension to avoid frequent temperature shift.

The digested sample can be added after all other reagents have been added. The maximum sample volume is 500 μL/well.

2. Program setting

a. Power button on→click "login" to enter account and password→enter the main page

b. Wipe the interior of the instrument with a 75% ethanol →click on "UV lamp" →select "15 minutes".

Note: This step can be set before the extraction preparation operation.

c. Place the 96-deep well plate in a fixed position in the instrument and insert the plastic sleeve into the corresponding position of the magnetic head.

d. Click "Run"→select "Virus-730" program→scan the two-dimensional code on the reagent kit→instrument working

e. At the end of the program, a "drip" sound is emitted. Immediately remove the deep well plate and transfer all the purified sample solution to the corresponding new 1.5 mL microfuge tube.

Precautions

1. It is recommended to separate molecular laboratory spaces for reagent preparation area (negative control sample preparation, PCR reagent preparation, negative test control preparation), sample preparation area (sample preparations), amplification area, etc. Each area clearly marked with a fixed sign and has

separate sets of equipment and supplies to avoid intermixing. Experimental reagents, test samples, and PCR amplification products should be stored separately and not in the same storage place. Eliminate every unnecessary walk in the experimental area to reduce the contamination risks.

2. Ensure that the ambient environment temperature not lower than 22°C during the experiment begins.

3. During the experiment, choose suitable gloves and change them regularly. Also use different lab coats, masks, hair covers and gloves in different experimental areas to avoid cross contamination.

4. Centrifuge the reagent tubes before opening to avoid aerosol production or liquid splashing, as well as to avoid contamination to the gloves or pipettes. The liquid on the caps or walls should be spun down to the bottom of the tube.

5. Used tips and liquid waste must be disinfected, and then discarded in a designated place, and if necessary, shipped off-site.

6. After PCR amplification, wear disposable gloves to take the PCR tube or plate out, and check whether the caps or seals are tightly closed, and whether the walls are cracked. Ensure that the reaction mixture does not leak. Discard it in the designated place, and the caps or seals should not be removed.

7. Place the tubes in the magnetic stand with the pellet against the magnet, and rotate the tubes slowly during the process to accelerate the magnetic particle aggregation.

8. During DNA washing and elution, centrifugation should be performed right after vortex to ensure that no magnetic particles or liquid on the tube caps or walls.

9. Do not over dry the pellet when removing the residual ethanol, over drying will make the pellets difficult to resuspend in the Elution buffer in next step.

10. Please perform DNA detection assay on the same day after DNA extraction to ensure the accurate results.

11. Before rHCDpurify program starts, ensure that the PCR plate and plastic sleeves are loaded appropriately.

12. Before and after rHCDpurify program running, UV sterilization of the machine is required for at least 15 min, and use 75% ethanol wipes to clean the insider walls. The minimum interval between two extractions is 30 min.

13. After rHCDpurify program, immediately transfer each sample solution to a new centrifuge tube. Condensed water may appear on the walls of Row 5th or 11th wells, which does not affect the DNA extraction, just simply transfer the bottom eluate to a new tube and guarantee more than 40μL as required for the assay.

Effective date: 08 Jul. 2024

Support & Contact

SHENTEK

Biofargo, Inc.
www.biofargo.com
Address: 1716 E Parham Rd Richmond, Va, 23228, USA
E-mail: contact@biofargo.com
Phone: 804-529-2296