GRS Viral DNA/RNA Purification Kit

#GK12.0100 (100 preps) | GK12s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	200 µl of cell-free medium (serum, body fluids, supernatant of viral infected cells), swab samples, sputum, and nasopharyngeal/ oropharyngeal/ tracheal washes/ aspirates
Expected Yield :	viral DNA and RNA (10^1-10^9 copies)
Format :	spin column (certified DNase/RNase-free)
Operation Time :	within 50 minutes
Elution Volume :	50µl

Product: The GRS Viral DNA/RNA Purification Kit provides an efficient and fast method for the purification of high-quality viral DNA and RNA from cell-free media (e.g., from serum, body fluids, and the supernatant of viral infected cell cultures). Eluted purified Nucleic Acid is suitable for all common applications, including PCR, real-time PCR, RT-PCR, One-step qRT-PCR, and DNA Sequencing. This kit is recommended for parallel purification of viral DNA (including CMV and HBV) and viral RNA (including HIV, HTLV, HCV, and SARS-CoV-2). The detection limit depends on the type of virus and on the sensitivity of individual PCR or RT-PCR protocols.

The GRS Viral DNA/RNA Purification Kit is optimized to eliminate the need of an internal control or carrier RNA. Lysis of DNA/RNA viruses using the Viral Lysis Buffer is fast and efficient. The buffer system is optimized to allow selective binding of nucleic acids to the glass fiber matrix of the spin-column[1]. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified DNA/RNA is subsequent eluted with RNase-Free Water. The entire procedure can be completed within 30 minutes without the use of phenol extraction.

QC: The quality of the GRS Viral DNA/RNA Purification Kit is tested on a lot-to-lot basis by isolating viral DNA/RNA form a 200 µl serum sample.

Caution: The Viral Lysis Buffer contains chaotropic salt, which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles. In order to prevent RNase contamination, one should use disposable plasticware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-Free and used only for RNA procedures. During handling, gloves should be worn at all times.

Safety: *Many viruses, including SARS-CoV-2, are pathogenic to human; hence isolation and identification must be carried out by trained laboratory personnel only, in a properly equipped laboratory with proper containment level. Care must be taken in the sterilization and disposal of all test materials. All procedures must be performed in the designated area of the laboratory. Disposable gloves and protective clothing must be worn during all procedures. All relevant National and Local Regulations must be met.*

Kit Contents	(100 preps)	(4 preps)
Viral Lysis Buffer	135 ml	2 ml
Binding Buffer*	8 ml	500 µl
Wash Buffer 1	50 ml	2 ml
Wash Buffer 2*	25 ml	1 ml
RNase-free Water	6 ml	1 ml
Viral DNA/RNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	200	4

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
PBS**
NALC-NaOH solution***

* Add Ethanol (96%-100%) [not included] to Binding Buffer and to Wash Buffer 2, as indicated on the bottles, prior to initial use. After Ethanol has been added, mark the bottles to indicate that this step has been completed. Close bottles tightly to avoid ethanol evaporation.

** PBS (Phosphate Buffered Saline) = 137mM NaCl, 2.7mM KCl,10mM Na_2HPO_4, and 2.0mM KH_2PO_4 pH7.4 (prepared with DNase/RNase-free water)

*** NALC-NaOH solution = 2% NaOH, 1.45% Na-citrate, 0.5% N-Acetyl-l-Cysteine (prepared with DNase/RNase-free water)

Storage: All components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C. Store for up to 2 years.

References: 1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

SAMPLE PREPARATION

General: For cell-free medium such as serum or supernatant of viral infected cell culture, as well as nasopharyngeal and oropharyngeal washes, follow the protocol for the purification of viral DNA and/or RNA starting with step 1.

Sputum samples and viscous nasopharyngeal/oropharyngeal/tracheal aspirates: Transfer into a sterile cup and add an equal volume of NALC-NaOH solution and mix with the specimen. Incubate at room temperature for 20 min with constant shaking. Centrifuge the liquefied sample to pellet debris, and transfer the clear supernatant to a clean tube. Transfer 200 µl of the lysate into a DNase/RNase-free 1.5-ml microcentrifuge tube. Then, proceed with step 2 of the main protocol by adding 400 µl of Viral Lysis Buffer and continue according to the protocol.

Alternatively, Sputum samples, can be liquefied with a DTT solution in PBS. For this, add 100 µl of 500mM DTT (dithiotheitol, not provided), freshly prepared in nuclease-free water, to 5 ml of cold sterile 0.01 M PBS (ph 7.2) and mix briefly. Transfer the sputum sample to a sterile cup and add an equal volume of diluted DTT and incubate at room temperature for 30 minutes with constant shacking. Discard any unused DTT solutions. Centrifuge the liquefied sample to pellet debris, and transfer the clear supernatant to a clean tube. Transfer 200 µl of the lysate into a DNase/RNase-free 1.5-ml microcentrifuge tube. Then, proceed with step 2 of the main protocol by adding 400 µl of Viral Lysis Buffer and continue according to the protocol.

Swab samples: Transfer the swab tip into a DNase/RNase-free 1.5-ml microcentrifuge tube containing 500 µl of Viral Lysis Buffer, and vortex for 30 seconds. Incubate at room temperature for 10 minutes. Remove the swab tip and proceed with step 4 of the main protocol by adding 450 µl of Binding Buffer and continue according to the protocol.

Swab samples collected in transport medium (e.g. VTM or PBS): Vortex the tube containing the swab tip that is immersed in transport medium for 20 to 30 seconds. Transfer 200ul of the medium to a DNase/RNase-free 1.5-ml microcentrifuge tube containing 400 µl of Viral Lysis Buffer, and vortex for 30 seconds. Incubate at room temperature for 10 minutes and proceed with step 4 of the main protocol by adding 450 µl of Binding Buffer and continue according to the protocol. Optionally, the swab tip may also be transferred to the Viral Lysis Buffer. In that case it should be removed just before adding 450 µl of Binding Buffer.

PROTOCOL FOR THE PURIFICATION OF VIRAL DNA & RNA

1) Transfer 200µl of cell-free medium (such as serum, plasma, body fluids, and supernatant of viral infected cell culture) into a DNase/RNase-Free 1.5-ml microcentrifuge tube.
If the sample volume is less than 200 µl, adjust volume with PBS (recipe on page 2).

2) Add 400 µl of Viral Lysis Buffer and mix by vortexing.

3) Incubate at room temperature for 10 minutes.

4) Add 450 µl of Binding Buffer (Ensure ethanol has been added) to the sample lysate and mix well by shaking vigorously.

5) Place the Viral DNA/RNA mini spin column in a 2-ml collection tube and transfer 600 µl of the lysate mixture to the column.

6) Centrifuge at 14,000g-16,000g for 1 minute. Discard the flow-through and place the column back in the collection tube. Transfer the remaining lysate mixture from step 4 to the column and centrifuge again at 14,000g-16,000g for 1 minute.

7) Discard the collection tube containing the flow-through and place the spin column in a new 2-ml collection tube.

8) Add 400 µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the column back in the collection tube. Add 600 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds (*Ensure ethanol was added to Wash Buffer 2, prior to use 1st time).

9) Discard the flow-through and place the spin column back in the collection tube and centrifuge for another 3 minutes at 14,000g-16,000g to dry the matrix of the column.

10) Transfer the spin column to a new 1.5-ml microcentrifuge tube (DNase/RNase-Free) and pipette 50µl RNase-Free Water directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3 minutes.

11) Centrifuge for 1 minute at 14,000g-16,000g to elute purified nucleic acid. Discard the spin column and use DNA and/or RNA immediately or store at -20°C/-80°C.