GRS Viral DNA/RNA Purification Kit – #GK12.050 (50 preps)

(FOR RESEARCH ONLY)

Sample : 200µl of cell-free medium (serum, plasma. body fluid, supernatant of viral infected cells)
Expected Yield : viral DNA and RNA (10^1-10^9 copies)
Format : spin column
Operation Time : within 30 minutes
Elution Volume : 50µl

Product Description

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, plasma, body fluids, and the supernatant from 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, and HCV). The detection limit depends on the type of virus and on the sensitivity of individual PCR or RT-PCR protocols.

Principle

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 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 subsequently eluted with RNase-Free Water. The entire procedure can be completed within 30 minutes without the use of phenol extraction.

Quality Control

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

Caution

The 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.

References

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

Kit Contents (100 preps)

Item	Quantity
Viral Lysis Buffer	30 ml
Binding Buffer*	4 ml
Wash Buffer 1	30 ml
Wash Buffer 2**	12.5 ml
RNase-Free Water	6 ml
Viral DNA/RNA mini spin column	50
2,0-ml collection tube	100
1,5-ml microtube (DNase/RNase free)	100

Required Components (not included)

Component
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips) (RNase-Free)
Vortex
Water bath or Thermoblock
PBS

Notes

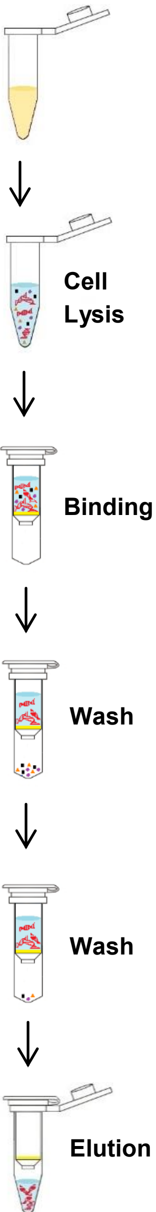
* Add 30 ml ethanol (96%-100%) [not included] to Binding Buffer prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.
** Add 50 ml ethanol (96%-100%) [not included] to Wash Buffer 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.

PBS (Phosphate-Buffered Saline) is 137mM NaCl; 2,7mM KCl; 4,3mM Na_2HPO_4; 1,47mM KH_2PO_4, pH 7.4, (prepared with DNase/RNase-free H_2O).

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 37ºC followed by cooling to 25ºC.

PROTOCOL FOR THE PURIFICATION OF VIRAL DNA & RNA



1) Transfer 200µl of cell-free medium (such as serum, plasma, body fluids, supernatant of viral infected cell culture) into a DNase/RNase free 1.5-ml microcentrifuge tube. If the sample volume is less than 200µl, adjust volume with PBS (recipe on page 2).
2) Add **400µl of Viral Lysis Buffer** and mix by vortexing.
3) Incubate **at room temperature** for 10 minutes.
4) Add **450µl of Binding Buffer** (check if 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 2,0-ml collection tube and transfer **600µl 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 2,0-ml collection tube.
8) Add 400µl 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 **600µl of Wash Buffer 2*** and centrifuge at 14.000g-16.000g for 30 seconds **(*check if ethanol is added first time the kit is used; see Notes on page 2)**.
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 pipet 50µl **RNase-Free Water** directly to the centre 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/RNA immediately or store at -20ºC.