Data sheet

Viral DNA/RNA Mini Spin Kit

Cat. No: AN0605 (100 reactions)

Description

Viral DNA/RNA Mini Spin Kit is designed for the rapid simultaneous purification of viral DNA and RNA from cell –free samples such as serum, plasma and cerebrospinal fluid.

The kit is based in nucleic acid ability to bind silica in the presence of high concentrations of chaotropic salts. The viral RNA/DNA molecules bind to the silica-based media and impurities such as proteins and nucleases are removed by thorough washing with Wash Buffer. The RNA/DNA is then eluted in sterile, RNase free water

Features

- **Safe**: no phenol-chloroform extraction.
- **Efficient**: 3-6 µg of genomic DNA from a 200 µl blood sample.
- **Ready to use** genomic DNA, in all molecular biology applications.

Applications

The purified viral RNA and DNA is suitable for use in RT-PCR, qRT-PCR, and qPCR, and can be used for:

- Viral load monitoring
- Viral detection
- Viral genotyping

Kit Storage:

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves, and allow to cool to room temperature before use.

Kit Components

Item	AN0605
Minispin columns	100
Collection tubes (2 mL)	200
BLY Buffer	25 ml
Proteinase K*	40 mg
WB1 Buffer**	36 ml
WB2 Buffer**	20 ml
Carrier RNA*** (lyophilized)	620 µg
Elution Buffer	15 ml

*Dissolve Proteinase K in water (1.5 ml) to obtain a 20 mg/mL stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

**Add the volume ethanol (96%-100%) specified [Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

*** Carrier RNA (620 µg) is delivered in lyophilized form. Dissolve Carrier RNA in RNase-free water to obtain a **stock solution (1 µg/µL)**: Add 620 µl RNase-Free $_{dd}H_2O$ to the tube containing 620 µg lyophilized. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Avoid repeated freezing and thawing.

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Before starting the protocol prepare the following reagent:

The purification protocol recommends using 5.6 µg Carrier RNA for sample

Addition of carrier RNA to Buffer BLY

Calculate the volume of **Buffer BLY/Carrier RNA mix** required to process the desired number of samples simultaneously using the following formula:

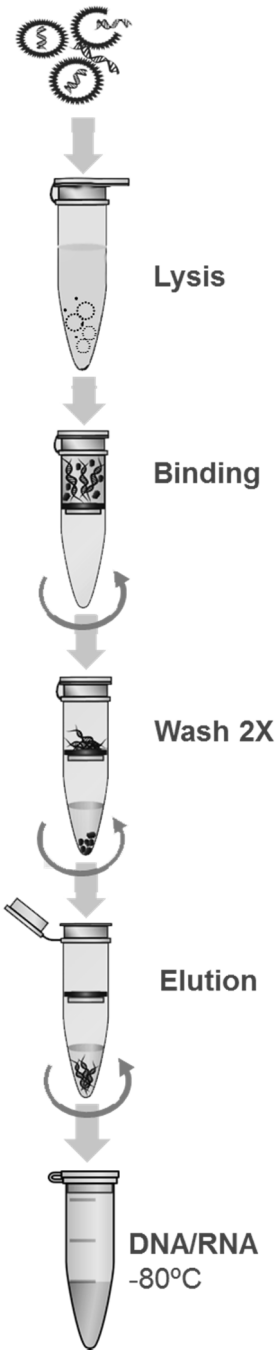
N × 0.21 mL = **X** mL

X mL × 28 µL/mL = **Y** µL

Where: **N** = number of samples; **X** = calculated volume of **Buffer BLY**; **Y** = calculated volume of 1 µg/µL **Carrier RNA stock solution** to add to **Buffer BLY**.

DETAILED PROTOCOL

1. Transfer 25µL proteinase K into the botton of a microcentrifuge tube.
2. Add 200 µL of sample (plasma or serum).
3. Add 200 µL buffer BLY (containing 5.6 µg of carrier RNA) and mix by vortexing vigorously for 20 seconds. If you are processing <200 µL sample, adjust final volume of the sample to 200 µL using PBS (phosphate buffered saline) or 0.9% NaCl.
4. Incubate in a water bath at 56 °C for 20 minutes.
5. Add 250 µl of ethanol (96–100%) and mix by vortexing vigorously for 15 seconds.
6. Incubate the lysate with the ethanol for 5 minutes at room temperature.
7. Transfer the mix to the minispin column by pipetting and centrifuge at 10.000rpm for 1 minute. Discard the flow-through.
8. Place the minispin column in a collection tube and add 500 µL of **buffer WB1**. Centrifuge at 12.000 rpm for 1 minute. Discard the flow-through
9. Place the minispin column in a collection tube and add 500 µL of **buffer WB2**. Centrifuge at 12.000 rpm for 3 minute. Discard the flow-through.
10. Place the minispin column in a collection tube and add 500 µL of **buffer WB2**. Centrifuge at 12.000 rpm for 3 minute. Discard the flow-through.
11. Centrifuge at full speed for an additional 3 min to dry the spin column.
    This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
12. Place the minispin column into a new, labelled 1.5 microcentrifuge tube and pipet 30-50 µL **Elution Buffer** directly into the membrane or RNase-free water. Close the cap and incubate for 1 minute at room temperature.
13. Centrifuge at full speed for 1 minute to elute. The eluate contains viral DNA and/ or viral RNA.
14. After extraction place the Elution Tube on ice. For long time storage place the nucleic acids at –80°C.



PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.