96-Well Viral DNA/RNA Kit

(Cat# K1417-1, -4; Store at MT)

I. Introduction:

BioVision's 96-Well Viral DNA/RNA Kit provides an easy and reliable method for isolating total viral DNAor viral RNA from whole blood in a 96-well format. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria. This procedure has been tested for isolating nucleic acids from Hepatitis A, Hepatitis C and HIV. The isolated DNA/RNA can be used for PCR, RT-PCR and other downstream applications

II. Sample Types:

- Blood

III. Kit Contents:

	K1417-1	K1417-4	
Components	**1 Plate**	**4 Plates**	**Part Number**
96-Well DNA/RNA Plate	1	4	K1417-XX-1
96-Well Deep Plates	2	4	K1417-XX-2
Buffer HLY	60 mL	200 mL	K1417-XX-3
Wash Buffer*	50 mL	50 mL X 2	K1417-XX-4
Protease K	1.1 mL	4.4 mL	K1417-XX-5
Carrier Nucleic acid	300 µL	1.2 mL	K1417-XX-6
Sealing Film	6	24	K1417-XX-7
DEPC Water	20 mL	40 mL	K1417-XX-8

Add 200 mL 100% ethanol to RNA Wash Buffer before use.

IV. User Supplied Reagents and Equipment:

- Centrifuge capable 4,000g with adaptor for 96-well plates at 4 -22°C.
- Vortex
- Multichannel pipet.
- RNase-free filter tips
- Reagent reservoirs for multichannel pipet
- RNase-free 1.2 mL 96-well plates
- 2 mL 96-well plates
- 100% ethanol

V. Shipment and Storage:

All components of the 96-Well Viral DNA/RNA Kit should be stored at 22°C -25°C except the Buffer HLY, Carrier, Protease K. Buffer HLY, Carrier and Protease K should be stored at 2-8°C. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

1. Add 200 mL 100% ethanol to RNA Wash Buffer before use.
2. Add all Carrier Nucleic acid to Buffer HLY. Transfer Carrier Nucleic acid to the bottle of HLY Lysis Buffer. Store at 4°C. Warm up Buffer HLY/carrier before use if precipitation forms at 4C。
3. Prepare Wash Buffer and HLY Lysis Buffer/carrier Nucleic acid solution as instructed.

VII. Viral DNA/RNA Protocol:

96-Well Viral DNA/RNA Protocol:

1. Add 150 µL plasma, acellular body fluid, cell culture supernatant to each well of a 1.2 mL 96-well plate (not provided).
2. Add 10 µL Protease K to each well.
3. Add 500 µL HLY Lysis Buffer/Carrier Nucleic acid solution to each well. Seal the plate with a Sealing Film.
4. Keeping the 96-well plate flat on the bench, shake vigorously back and forth for 30 seconds. Rotate the plate 90° and shake the plate for another 30 seconds.
5. Let sit at room temperature for 10 minutes.
6. Briefly centrifuge at 500g to collect any liquid droplets from the film.
7. Remove and discard the Sealing Film.
8. Add 350 µL 100% ethanol to each well. Seal the plate with a Sealing Film.
9. Vortex the plate for 30 seconds. Briefly centrifuge at 500g to collect any liquid droplets from the film. Remove and discard the Sealing Film.
10. Place a 96 Well Plate onto a 96-well Deep Plate (provided).
11. Transfer 500 µL sample from Step 8 (including any precipitate that may have formed) to each well of the 96 Well Plate.
12. Seal the 96 Well Plate with a Sealing Film.
13. Centrifuge at 4,000g for 5 minutes at room temperature.
14. Discard the filtrate from the 96-Well Deep Plate.
15. Remove and reuse the Sealing Film in the following step.
16. Repeat Steps 11-15 until the entire sample has been transferred to the 96 Well Plate.
17. Remove the Sealing Film.
18. Add 750 µL Wash Buffer to each well. Seal the plate with the Sealing Film. Place the 96 Well Plate onto the 96-well Deep Well Plate.
19. Centrifuge at 4,000g for 10 minutes at 4°C. Discard the flow through and centrifuge the plate at 4000xg for 10 min at 4°C. *Note: It is important to dry the 96 Plate matrix before elution. Residual ethanol may interfere with downstream applications*.
20. Remove and discard the Sealing Film. Transfer the 96 Well Plate to an Elution Plate.

21. Add 100-150 µL DEPC-treated water to each well. Seal the plate the new Sealing Film. Make sure to add water directly onto RNA matrix.
22. Let sit for 1 minute at room temperature.
23. Centrifuge at 4,000g for 5 minutes at 4°C. The RNA/DNA is in the flow through.
24. Store RNA/DNA at -80°C.

VIII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Little or no Nucleic acid eluted	• Carrier Nucleic acid Solution not added to HLY Buffer or degraded • RNA remains on the plate • Plate is overloaded	• Dissolve the carrier solution with HLY Lysis Buffer and repeat the purification with a new sample. • Avoid warming the HLY/carrier solution. • Repeat elution. • Preheat DEPC Water to 70°C prior to elution. • Let sit for 5 minutes with DEPC Water prior to elution • Reduce the amount of starting material.
Clogged well	• Incomplete lysis	• Mix thoroughly after addition of HLY Lysis Buffer. • Reduce the amount of the starting material.
Degraded RNA	• Source • RNase contamination	• Do not freeze/thaw sample more than once. • Follow protocol closely and work quickly. • Low concentration of virus in the sample. • Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	• Salt carryover during elution • Inhibitors of PCR	• Ensure Wash Buffer has been diluted with 100% ethanol as instructed. • Wash Buffer must be stored at room temperature. • Repeat wash with Wash Buffer. • Use less starting material. • Increase incubation with HLY Lysis Buffer to completely lyse the cells.
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 minutes.

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