E.Z.N.A.® Viral RNA Kit

Revision No: 2.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	R6874-00	R6874-01	R6874-02
Purifications	5	50	200
HiBind® RNA Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
QVL Lysis Buffer	5 mL	30 mL	120 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
VHB Buffer	4.4 mL	22 mL	66 mL
Carrier RNA	1 mg	1 mg	1.5 mg
Nuclease-free Water	2 mL	30 mL	60 mL

Supplied by user:

- Microcentrifuge capable of at least 13,000*g*
- 100% ethanol
- Sterile nuclease-free 1.5 mL microcentrifuge tubes
- Sterile nuclease-free pipette tips

Before starting:

- Equilibrate samples and QVL Lysis Buffer to room temperature
- Prepare RNA Wash Buffer II and VHB Buffer according to the directions on the bottles
- Prepare Carrier RNA according to the table below

Prepare Carrier RNA solution. Add Nuclease-free Water to the lyophilized Carrier RNA according to the table below. Dissolve the Carrier RNA completely, aliquot, and store at –20°C. Do not freeze–thaw the aliquots more than three times.

Product	Amount of Nuclease-free Water
R6874-00	1 mL
R6874-01	1 mL
R6874-02	1.5 mL

RNA Extraction and Purification from Cell-free Fluids

LYSE

1. Prepare a mastermix of QVL Lysis Buffer and Carrier RNA. Use the table below for guidance.

Number of Preps	Amount of QVL Lysis Buffer (mL)	Amount of Carrier RNA (µL)
1	0.56	5.6
2	1.12	11.2
3	1.68	16.8
4	2.24	22.4
5	2.80	28.0
6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0

Note: QVL Lysis Buffer and Carrier RNA mastermix is stable at 2-8°C for 48 hours. When stored at 2–8°C, this mixture forms a precipitate that must be redissolved before use. Warm the mixture to 80°C. Do not warm for more than 5 minutes.

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LYSE

2. Add 500 µL mastermix from Step 1 into a 1.5 mL microcentrifuge tube (not provided).
3. Add 150 µL plasma, acellular body fluid, cell culture supernatant, or urine to the mastermix. Vortex for 30 seconds to mix thoroughly.
4. Let sit at room temperature for 5-10 minutes.
5. Centrifuge briefly to collect any liquid droplets from the lid.

BIND

6. Add 350 µL 100% ethanol. Vortex for 30 seconds to mix thoroughly. Centrifuge briefly to collect any liquid droplets from the lid.
7. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.
8. Transfer 750 µL sample (including any precipitate) to the HiBind® RNA Mini Column.
9. Centrifuge at maximum speed (≥13,000*g*) for 15 seconds. Discard the filtrate and reuse the collection tube.
10. Repeat Steps 8-9 until all the sample has been transferred to the HiBind® RNA Mini Column.

WASH

11. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.
12. Add 500 µL VHB Buffer diluted with 100% ethanol (see bottle for instructions). Centrifuge at maximum speed for 15 seconds. Discard the filtrate and the collection tube.
13. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.
14. Add 500 µL RNA Wash Buffer II diluted with 100% ethanol (see bottle for instructions). Centrifuge at maximum speed for 15 seconds. Discard the filtrate and reuse the collection tube.
15. Repeat Step 14 for a second RNA Wash Buffer II wash step.
16. Centrifuge the empty HiBind® RNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

17. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).
18. Add 20-50 µL Nuclease-free Water directly to the center of the column matrix.
19. Centrifuge at maximum speed for 1 minute.
20. Store RNA at -70°C.