FOR RESEARCH USE ONLY!

Viral RNA Extraction Kit for Respiratory Specimens

Rev 12/20

(Catalog # K1462-50, -250; 50 or 250 Rxns; Store at Multiple Temperatures)

I. Introduction:

BioVision's Viral RNA Extraction Kit for Respiratory Specimens provides an easy and reliable method for isolating total viral RNA from plasma, serum, nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates and sputum using spin columns. This procedure has been tested for isolating nucleic acids from Hepatitis A, Hepatitis C and HIV. The isolated RNA can be used for PCR, qRT-PCR and other downstream applications. BioVision offers the Viral RNA Extraction Kit to facilitate research on Viruses.

II. Application:

- An ideal tool to extract viral RNA from respiratory specimens

III. Key Features

- Rapid, easy and convenient
- **Highly pure**, high yield
- Extract Viral RNA for **many downstream applications**
- High quality spin columns

IV. Sample Types:

- Plasma, serum, nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates and sputum.

V. Kit Contents:

Components	K1462-50 (50 Rxns)	K1462-250 (250 Rxns)	Part Number
Buffer LY	20 ml	90 ml	K1462-XX-1
L Solution	120 µl	550 µl	K1462-XX-2
Proteinase K	1.1 ml	4 ml	K1462-XX-3
RNA Wash Buffer *	12 ml	50 ml	K1462-XX-4
Buffer RB	20 ml	90 ml	K1462-XX-5
DEPC-Treated Water	10 ml	15 ml	K1462-XX-6
RNA Column	50	250	K1462-XX-7

RNA Wash Buffer must be diluted with 100% ethanol before starting. Add 48 ml (K1462-50) or 200 ml (K1462-250) to RNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

VI. User Supplied Reagents and Equipment:

- Pipettes
- Pipette tips
- 100% Ethanol
- β-mercaptoethanol
- DEPC treated water (RNase/DNase free)
- PBS
- RNase free 1.5 ml microcentrifuge tubes
- Microcentrifuge

VII. Shipping and Storage Conditions:

The kit is shipped at Room Temperature (RT). Proteinase K and L solution should be stored at -20 °C. All other reagents should be stored at RT. The kit reagents will be stable for 12 months if stored properly.

VIII. Reagent Preparation and Storage Conditions:

1. RNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1462-50) or 200 ml (K1462-250) to RNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Aliquot Buffer LY into a clean tube and add β-mercaptoethanol to a final concentration of 1% in Buffer LY. Then add 4 µl of L Solution to 1 ml of Buffer LY/β-mercaptoethanol solution. Mix well.
3. Buffer LY and Buffer RB contain chaotropic salts, wear gloves and protection eyewear when handling these buffers.

IX. Viral RNA Isolation:

This protocol is developed for a sample volume of 150 µl. Smaller sample volumes should be adjusted to 150 µl with PBS before loading and samples with a low viral titer should be concentrated to 150 µl before processing. For sample volumes of 150 µl-300 µl, the volumes of Buffer LY and other reagents should be increased proportionally, but the volumes of Buffers RB and RNA Wash Buffer used in the wash steps need not be increased.

1. Prepare a master mix containing **Buffer LY, β-mercaptoethanol and L Solution** as described in the Reagent Preparation section. The mixture of Buffer LY and RNA carrier is stable at 2-8 ℃ for 48 hr.
2. Pipet **150 µl plasma, serum, cell free body fluid or other samples** into a 1.5 ml tube and add **0.35 ml of Buffer LY/β-mercaptoethanol/L Solution**.
3. Add **15 µl Proteinase K** and mix thoroughly by vortexting. Incubate at 30°C for 20 min. Proteinase K is necessary for extracting RNAs from nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, and sputum.
4. Add **1 volume of 100% ethanol** into the lysate and pipet 5 times to mix the solution.

5. Transfer the solution into a **Mini Column** and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the column back in the microcentrifuge tube.
6. Add **350 µl Buffer RB** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through. Put the column back in the microcentrifuge tube.
7. Add **500 µl RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 sec. Discard the flow-through. Put the column back in the microcentrifuge tube.
8. Centrifuge the **empty column** at 12,000 rpm for 2 min. This step is critical to remove the residual ethanol for optimal elution.
9. Place the column in an RNase-free 1.5 ml tube and add **35-50 µl DEPC-Treated Water** to the column. Centrifuge at 12,000 rpm for 1 min. The viral RNA is in the flow-through liquid. Store the purified RNA at -20°C
10. Optional step: Add the eluent back to the column for a second elution. *Note: The first elution normally yields 60-70% of the RNA while the second elution will yield another 20-30% of the DNA/RNA bound to the column.*

X. General Troubleshooting Guide:

Problem	Possible reason	Suggested Improvement
Low A_{260}/A_{280} ratio	Protein contamination	Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low A_{260}/A_{280} ratio	Guanidine Thiocyanate contamination	Add 2.5 volumes of ethanol and 0.1 M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20°C. Centrifuge at 10,000 g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	RNA in sample is degraded	Freeze samples immediately in liquid nitrogen and store at -70°C after collection.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Adding too much sample exceeding the binding capacity of spin column will decrease the total RNA yield.
Low Yield	Ethanol not added to buffer	Add ethanol to the Wash Buffer.
Genomic DNA contamination	Too much total RNA sample was used in RT-PCR	Reduce the amount of total RNA used in RT-PCR to 50-100 ng.

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
Coronavirus (SARS-CoV-2) PCR Detection Kit	K1461	100 Rxns
EasyRNA™ Blood RNA Mini Kit	K1373	50, 250 Preps
EasyRNA™ Cell/Tissue RNA Mini Kit	K1337	50, 250 Preps
EasyRNA™ Bacterial RNA Kit	K1351	50, 250 Preps
EasyRNA™ Fungal RNA	K1419	50, 250 Preps
EasyRNA™ Plant RNA Mini Kit	K1374	50, 250 Preps
miRNA Extraction Kit	K1456	50 preps
Mag-Lentivirus and Retrovirus Purification Kit	K1458	20, 100 Preps
PEG Virus Precipitation Kit	K904	50, 200 Preps
Viral DNA Extraction Kit	K1446	100 Preps
Yeast RNA Mini Kit	K1418	50, 250 Preps
96-well Viral DNA/RNA Kit	K1417	1, 4 Plates

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