Life Technologies™
Your Molecular & Cell Technology Partner

Life Technologies User Bulletin

AuPreP Viral RNA Extraction Miniprep System

For biological fluids containing RNA virus: serum, plasma, body fluids, and cell culture supernatant.

Downstream Application

- Northern, dot and slot blotting
- RT-PCR / Quantitative real-time PCR
- Poly A^+ RNA selection
- cDNA Synthesis/ Primer extension
- Array analysis
- *In vitro* translation
- NGS
- Gene editing

Product Contents

Cat Number .	RNV-52-904LT	RNV-52-906LT
Preps	50	250
RXV Buffer	35ml	190ml
WS Buffer	15ml	45ml x 2
RNA Carrier	1	1
Proteinase K	10mg	3x10mg
RNase-free ddH_2O	6ml	27ml
RNA Mini Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Life Tech Viral RNA Extraction System is shipped at ambient temperature and stored for up to 6 months.

If precipitation forms by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

- **Please read the following notes before starting the procedure.**

WARNING, strong acids and oxidants (for instance, bleach) should not be used together with RXV buffer (because this kind of reaction would produce toxic cyanide)!!!

Important Notes

- Preheat RNase-free ddH_2O for elution to 80°C.

-Add 1 ml sterile ddH_2O to each tube to reconstitute the provided Proteinase K by vortexing. Store this Proteinase K solution at 4℃.

-For RNV-52-904LT: Add 60 ml of ethanol (98-100%) to the WS Buffer bottle when it is opened for the first time.

-For RNV-52-906LT Add 180 ml of ethanol (98-100%) to the WS Buffer bottle when it is opened for the first time.

Except for the centrifuge steps done at the speed indicated in the protocol, all other centrifuge steps are to be done at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge.

The EasyLid is designed to prevent contamination during the procedure.

Twist the arm of the cap and pull the cap to break the EasyLid.

1. Add RNA carrier to RXV Buffer.

Add 1 ml RXV Buffer to the RNA carrier tube, vortex to dissolve and transfer to the RXV Buffer bottle, store at 4°C.

2. Pipet 150 µl sample (serum, plasma, body fluids, and cell culture supernatant) into a 1.5 ml tube.

3. Add 570 µl of carrier added RXV Buffer to the sample, mix by vortexing.

Through mixing is required for sample lysis. If the sample volume is larger than 150 µl, increase the amount of RXV buffer proportionally.

4. Add 10 µl Proteinase K to the sample and incubate at 50°C for 10 minutes.

5. Add 570 µl of ethanol (98-100%) to the sample, and mix by vortexing.

If the starting sample is larger than 150 µl, increase the amount of ethanol proportionally.

6. Place a RNA Column in a 2 ml Collection Tube, apply 650 µl of the ethanol added sample from step 5 to the RNA Column, close the cap, centrifuge at 6,000 x g (8,000 rpm) for 1 minute, and discard the filtrate.

If the solution remains above the membrane, centrifuge again at 13,000 rpm.

7. Repeat step 6 for rest of the sample.

8. Wash the column twice with 500 µl of ethanol added WS Buffer by centrifuging at full speed (13,000 rpm or 10,000 x g) for 1 minute, and discard the filtrate.

Add 60 ml (for RNV-52-904LT) or 180ml (for RNV-52-906LT) of ethanol (98-100%) to the WS Buffer bottle when the bottle is opened for the first time.

9. Centrifuge at full speed for 5 minutes to remove traces of WS Buffer.

Residual ethanol may cause the low A_{260}/A_{230} and inhibit reverse transcriptase activity.

10. Transfer the column to a RNase-free 1.5 ml tube (not provided), add 50 µl of preheated (80°C) RNase-free ddH_2O to centre of membrane, wait 1 minute and then centrifuge at full speed for 1 minute to elute RNA in tube. Again add 50ul of preheated (80°C) RNase-free ddH2O to column, wait 1 minute and centrifuge at full speed for 1 minute to elute RNA. Thus, a total of 80 to 100ul elute containing RNA is now available for downstream applications after the the two elutions.

11. Store eluted RNA at -70°C.

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25th May 2020

Dr. Hemant Sahni
Ms. Life Technologies

Subject: Performance Evaluation Report for AuPreP viral RNA extraction miniprep system (Lot No. LT-VRM-18), Life Technologies

Sir

We have evaluated the AuPreP viral RNA extraction miniprep system (Lot No. LT-VRM-18), Life Technologies. The final report is attached for your information.

Yours sincerely

Prof. Priya Abraham

Director

विश्व स्वास्थ्य संघटन
उभरते वायरल संक्रमणों का सहयोग केन्द्र
राष्ट्रीय शीतज्वर केन्द्र
पोलिओ, खसरा एवं रुबेला के लिए रेफरल प्रयोगशाला

WORLD HEALTH ORGANIZATION
Collaborating Centre for Emerging Viral Infections
National Influenza Centre
Referral Lab for Polio, Measles and Rubella

Evaluation report for RNA extraction kit

Objective: To evaluate the performance of RNA extraction kit using a panel of SARS-CoV-2 positive and negative samples.

Name of the Kit: AuPreP viral RNA extraction miniprep system (Lot No. LT-VRM-18), Life Technologies

Kit components: RXV buffer, WS Buffer, RNA carrier, Proteinase K, RNase free ddH_2O, RNA mini columns, collection tubes

Other requirements: Ethanol

Sample Panel: Fifteen SARS-CoV-2 positive samples and five SARS-CoV-2 negative samples

Methodology: RNA was extracted from the SARS-CoV-2 positive samples and negative samples according to the manufacturer's instructions and subjected to SARS-CoV-2 screening and confirmatory assays targeting E, ORF1b and RdRP genes and RNAseP internal control as per the ICMR-NIV SARS-CoV-2 RT-qPCR protocol.

Results:
Among the positive samples, all the samples gave positive result. Among the negative samples, RNA from all samples gave negative results. RNAseP (housekeeping gene) amplification was seen in RNA extracted from all 20 samples.

Conclusion:
The performance of the AuPreP viral RNA extraction miniprep system (Lot No. LT-VRM-18) is considered as SATISFACTORY

Note:
This evaluation report is exclusively for AuPreP viral RNA extraction miniprep system (Lot No. LT-VRM-18) Life Technologies

The company shall not publish or use information related to this evaluation without prior written consent from ICMR-NIV.

Prof. Priya Abraham

Director

ICMR-NIV

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