***Note**: for laboratory research use only*

Viral RNA Extraction Kit

A kit for fast isolation of viral RNA from whole blood, serum, plasma, oropharyngeal swab, and other biological fluids

Cat. #: RP9001（50 preps）

Signalway Biotechnology

I. Kit Content, Storage, and Stability

Content	Storage	50 preps (RP9001)
Lysis Buffer: VLS	4℃	40 ml
Carrier RNA	-20℃	200 μl
Washing Buffer: RW	4℃	15 ml
		Add 60ml ethanol before first-time use
Buffer RH	RT	9 ml
		Add 21ml ethanol before first-time use
RNase-free H_2O	RT	10 ml
RNase-free Tubes (1.5ml)	RT	50
RNase-free Tubes (2ml)	RT	50

All reagents are stable for 12 months when stored properly.

Reminder:

1. ***Add ethanol to Buffer RW and Buffer RH before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

II. Principle:

This product applies for the isolation of viral RNA from whole blood, serum, plasma, oropharyngeal swab, and other biological fluids. Carrier RNA has been used in this kit. Carrier RNA not only enhances precipitation of viral RNA, but also reduces RNA degradation.

III. Features:

1. Rapid and convenient. Experiments can be done in 30-40 minutes and achieve high quality of viral RNA with no contamination of DNA and proteins.
2. Do not contain poisonous phenol and does not require ethanol precipitation.
3. Pure RNA in high quality can be applied for RT-PCR, real-time qPCR, Northern blot, Dot blot, poly(A)+ selection, in vitro translation, RNase protect analysis, and molecular cloning**.** In addition, Lysis Buffer VLS is a great storage solution for viral RNA. Viral lysate (add Lysis Buffer VLS to sample based on the volume ratio 3:1 and vortex rigorously) can be stored up to 2 months at -20℃ and half year at -70℃; the lysate can be stable up to 1 day at 4℃ and 1 week at -20℃ during transportation.

IV. Notes

Please read this section before your experiment.

1. **To prevent RNA degradation, all the centrifugation steps should be performed under 4℃ unless being specially noted**. Recommend to use traditional centrifuge (up to 13,000 rpm), such as Eppendorf 5415C.
2. Reagent and buffer may contain a corrosive compound; wear latex gloves to avoid direct contact with skin, eyes, and clothes**. If contact occurs, wash with water or physiological saline.**
3. Due to the prevalence of RNases, wear gloves during the procedure and change them whenever contact by reagents occurs; please follow standard laboratory procedures of "Molecular Clone" rules.

   * *Wear gloves during entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.*
   * *Use sterile, disposable plastic-ware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any*

non-disposable items (such as automatic pipettes) can be rich sources of RNases.

** Treat non-disposable glassware and non-disposable plastic-ware to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic-ware with 0.1N NaOH with 1mM EDTA followed by RNase-free water.*

4. The routine method to determine the yield and purity of RNA is spectrophotometry (OD_{260}/OD_{280}). Please dissolute RNA by TE. Water will make OD_{280} higher because of lower ion intensity and pH.
5. The sample, which was mixed with Buffer MRL and was homogenized (without chloroform), can be stored under –70℃ for half year.
6. Chloroform (without any additives, such as isoamyl alcohol) should be prepared by user.

V. Procedure

Before Starting

- *Add carrier RNA to Lysis Buffer VLS (4 µl carrier per 750 µl VLS).*
- *Precipitate may occur in Buffer VLS under low temperature. Incubate at 37°C for a few minutes until buffer is clear, and then let the buffer cool to RT before use*
- *Add absolute ethanol into Buffers RW and RH, vortex adequately, then mark the check box to avoid multiple adding!*

1. Pipet 750 µl Lysis Buffer VLS (carrier RNA has been added) to a 1.5 ml RNase-free microcentrifuge tube.

   If the sample volume is larger than 250 µl, increase the amount of Lysis Buffer VLS-carrier RNA

proportionally (e.g., a 500 µl sample requires 1500 µl Lysis Buffer VLS).

Generally, plasma, serum, oropharyngeal swab, and other biological fluids often have low viral titers. Hence, concentrating these samples is recommended. Please follow the manufacturer's instructions to concentrate samples to the final volume 250 µl.

2. Add 250 µl whole blood or serum, plasma, oropharyngeal swab, and other biological fluids to the Lysis Buffer VLS-carrier RNA in the microcentrifuge tube. Vortex for 2 minutes to mix thoroughly.

   The lysate can be used for RNA isolation immediately or can be stored up to 2 months at -20℃ and half year at -80℃.

3. Incubate for 10 minutes at RT.
4. Add 150 µl chloroform and shake tube vigorously for 15 seconds, and incubate for 3 minutes at RT.
5. Centrifuge the samples at 12,000 rpm for 10 minutes at 4℃.The mixture separates into 3 phases: an upper aqueous phase, interphase, and a lower phenol-chloroform phase. RNA remains in the upper aqueous phase. The volume of aqueous phase is around 70% of Lysis Buffer VLS for initial homogenization.
6. Transfer the aqueous phase (about 600 µl) to an RNase-free tube (2 ml), and add 1.2 ml 100% ethanol, and incubate at RT for 10 minutes. Centrifuge at 12,000 rpm for 10 minutes at 4℃.

   The Volume of 100% ethanol should be about 2-fold that of aqueous phase and may be about one and half that of the Lysis Buffer VLS for initial homogenization.

7. Remove the supernatant; add 700 µl Washing Buffer RW, vortex and centrifuge at 12,000 rpm for 1 minute. Discard the supernatant.

   Add 1 ml Buffer RH, vortex 10 seconds and centrifuge at 12,000 rpm for 1 minute. Discard the supernatant.

8. Air-dry RNA pellet for 5-10 minutes. Dissolve it with 10-30 µl RNase-free H_2O.

   It's very important to dry RNA pellet completely. Otherwise, solubility of RNA pellet will be decreased.

9. Store RNA in -20℃ or apply to down-stream reactions.

VI. Troubleshooting

Problem	Possible Cause	Possible Solution
Little or no RNA in the elute	Carrier RNA not added	Add carrier RNA
	Carrier RNA was degraded	Maintain Lysis Buffer VLS-carrier RNA at -20°C
	Sample was frozen and thawed more than once	Aliquots to prevent repeated freeze-thaw
	Low viral titers	Concentrate samples before lysis
	Lysis Buffer VLS prepared incorrectly	Check VLS for precipitate; incubate in 37°C water bath to dissolve it.
	RNA degraded	Process samples quickly and add RNA inhibitor if necessary
DNA present in the elute	DNA contamination	Use DNA enzyme to digest