

Viral RNA Extraction Kit

RNP 9001 (50 preps)

Kit Content, Storage Condition and Stability

Content	Storage	50 preps
Lysis Buffer VLS	4°C	40 ml
Carrier RNA	-20°C	200 ul
Washing Buffer RW *	4°C	30 ml
70% ethanol *	RT	9 ml
RNase-free H ₂ O	RT	10 ml
RNase-free Tubes 1,5 ml	RT	50
RNase-free Tubes 2 ml	RT	50

All reagents, when stored properly, are stable for 12 months.

* Add the appropriate volume of 100% ethanol to Washing Buffer and 70% ethanol bottles separately based on the label instruction before use.

Description:

Viral RNA Extraction Kit is a fast reagent 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. Experiments can be done in 30-40 minutes and achieve high-quality viral RNA with no contamination of DNA and proteins. RNA 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°C and half year at -80°C; also the lysate can be stable up for 1 day at 4°C and 1 week at -20°C in transportation.

Materials required, not provided with kit

Chloroform (**without any additives**, such as isoamyl alcohol)

100% ethanol

Note:

To prevent RNA degradation, all the centrifuge steps should be made under 4°C, except having special notes.

Due to the prevalence of RNases, wear gloves at all times and change them whenever may have been contacted by reagents, please follow standard laboratory procedures of "Molecular Clone" rules.

* Wear gloves in entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.

* Use sterile, disposable plasticware 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 nondisposable items (such as automatic pipettes) can be rich sources of RNases.

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

The most common method to determine the yield and purity of RNA is spectrophotometry (OD260/OD280). Please dissolve RNA by TE, water will make OD280 higher because of lower ion intensity and pH.

Procedure

WARNING: When working with reagents, use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing.

Use in a chemical fume hood. Avoid breathing vapor. Unless otherwise stated, the procedure is carried out at 15 to 30°C.

Preparation of reagents

1. Add the appropriate volume of 100% ethanol to Washing Buffer and 70% ethanol bottles separately before use.
2. Check all solutions for precipitate and if necessary incubate at 37°C water bath for several minutes until precipitate is dissolved.

Protocol

1. Pipet 750 µl Lysis Buffer VLS + 4 µl carrier RNA 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 will require 1500 µl Lysis Buffer VLS).
Generally, plasma, serum, oropharyngeal swab and other biological fluids often have low viral titer. Hence, concentration of 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 min to thoroughly mix.
The lysate can be used for RNA isolation immediately or can be stored up to 2 months at -20°C and half year at -80°C; also the lysate can be stable up for 1 day at 4°C and 1 week at -20°C in transportation.
3. Incubate for 10 min at RT.
4. Add 150 µl chloroform and shake tube vigorously for 15 sec and incubate for 3 min at RT.
5. Centrifuge the samples at 12,000 rpm for 10 min at 4°C.
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 (around 600 µl) to a RNase-free tube (2 ml), add 1.2 ml 100% ethanol, incubate at RT for 10 min and then centrifuge at 12,000 rpm for 10 min at 4°C.
The Volume of 100% ethanol should be around 2-fold that of aqueous phase and may be around 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 min and discard the supernatant.
8. Add 1 ml 70% ethanol, vortex 10 sec and centrifuge at 12,000 rpm for 1 min and then discard the supernatant.
9. Air-dry RNA pellet for 5 - 10 min. Dissolve it with 10-30 µl RNase-free H₂O.
It's important not let RNA pellet dry completely because this will decrease its solubility.

Troubleshooting:

Problem	Possible Cause	Possible Solution
Little or no RNA in the eluate	Carrier RNA not added	Add carrier RNA
	Degraded carrier RNA	Maintain Lysis Buffer VLS-carrier RNA at -20°C
	Sample frozen and thawed more than once	Frozen and thawed more than once can degrade RNA
	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 eluate	DNA contamination	Recommend use cell-free samples