Note: For laboratory research use only.

Viral RNA Extraction Kit

Cat. # RP 9001 50 preps

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Procedure

CAUTION: 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

Add carrier RNA to Lysis Buffer VLS (4μL carrier per 750μL VLS).

Add add the appropriate volume of 100% ethanol to Washing Buffer and 70% ethanol bottles separately before use.

Check all solutions for precipitate, and if necessary incubate at 37℃ water bath for several minutes until precipitate is dissolved.

1. **Pipet 750μL Lysis Buffer VLS (carrier RNA has been added) to a 1.5mL RNase-free microcentrifuge tube.**
   If the sample volume is larger than 250μL, increase the amount of Lysis Buffer VLS-carrier RNA proportionally (e.g., a 500μL sample will require 1500μL Lysis Buffer VLS).
   Generally, plasma, serum, oropharyngeal swab, and other biological fluids often have low viral titers. Hence, to concentrate these samples is recommended. Please follow the manufacturer's instructions to concentrate samples to the final volume 250μL.
2. **Add 250μL whole blood or serum, plasma, oropharyngeal swab, and other biological fluids to the Lysis Buffer VLS-carrier RNA in the microcentrifuge tube. Vortex for 2min to thoroughly mix.**
   The lysate can be used for RNA isolation immediately or store up can be stored up to 2 months at -20℃ and half year at-80℃; also the lysate can be stable up for 1 day at 4℃, and 1 week at -20℃ in transportation.
3. **Incubate for 10min at RT.**
4. **Add 150μL chloroform and shake tube vigorously for 15sec, and incubate for 3min at RT.**
5. **Centrifuge the samples at 12,000 rpm for 10min 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 (around 600μl) to a RNase-free tube(2mL), add 1.2mL 100% ethanol, incubate at RT for 10min, and then centrifuge at 12,000rpm for 10min at 4℃.**
   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 700μl Washing Buffer RW, vortex and centrifuge at 12,000 rpm for 1min, and discard the supernatant.**
8. **Add 1mL 70% ethanol, vortex 10sec and centrifuge at 12,000rpm for 1min, and then discard the supernatant.**
9. **Air-dry RNA pellet for 5-10min. Dissolve it with 10-30μl RNase-free H_2O. It's important not let RNA pellet dry completely because this will decrease its solubility.**

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