PrimePrep™ Viral RNA/DNA Extraction Kit

Introduction

***PrimePrep*™ Viral RNA/DNA Extraction Kit** is provide a fast, easy method for the preparation of viral RNA and DNA from plasma, serum, cell-free bo dy fluids, cell-culture supernatants and virus-infected samples. *PrimePrep*™ Viral RNA/DNA Extraction Kit buffer system provides the effective binding c ondition of RNA and DNA to microfiber-silica-based membrane through m ix with lysis and binding buffers. And then the impurities on the membrane are washed away by two different wash buffers. Purified viral RNA/DNA re ady for use in downstream applications such as PCR, RT-PCR, cDNA synthe sis and real-time PCR etc.

Kit Components

Cat. No. / Reagents	KR-2000 (50 Prep.)
Spin column	50 ea
Collection tube	100 ea
Buffer VRL	40 ml
Buffer VRW1	20 ml
Buffer VRW2	11 ml
Buffer VRE	10 ml

Before you begin

- **Add 20 ml absolute ethanol to Buffer VRW1 before use.**
- **Add 44 ml absolute ethanol to Buffer VRW2 before use.**
- **Prepare β-mercaptoethanol (14.2 M), isopropanol, 1.5 ml microcentrifuge tube.**

! Caution:
- This product is for research use only.
- Buffer VRL and Buffer VRW1 contains strong denaturant. Be careful to avoid co ntacting with skin and eyes. In the case of such contact, wash immediately wi th plenty water.

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Experimental Protocol

1. **Prepare 200 µl of sample (plasma, serum, cell-free body fluids, cell-culture supernatants, virus-infected samples) into a 1.5 ml microcentrifuge tube.**
   · If the sample volume is larger than 200 µl, increase the amount of Buffer VRL (e.g. a 400 µl sample will require 700 µl Buffer VRL).

   √**Note**: Virus-infected samples are infected feces and cell line.
   In the case of feces samples, transfer 20~50 mg in 1.5 ml microcentrifuge tube and dissolve with 250 µl of DEPC-DW and vortex for 10~15 sec.
   Centrifuge at 13,000 rpm for 30 sec at room temperature. Use approximately 150~200 µl of supernatant.

2. **Add 350 µl of Buffer VRL and then add 3.5 µl β-mercaptoethanol (β-ME, 14.2 M). Mix by vortexing for 10~15 sec.**
   · Check Buffer VRL for precipitation. If happened, dissolve precipitate completely by incubation at 40~50 °C until dissolve.
   · β-ME must be add to Buffer VRL as ratio of 10 µl of β-ME per 1 ml of Buffer VRL.

3. **Incubate at room temperature (15~25 °C) for 10 min. After this step, cen trifuge at 3,000 rpm for 5 sec at room temperature.**
   · For protect from contamination by carryover, spin-down the tube to remove dr ops from the inside of the lid.

4. **Add 150 ㎕ of isopropanol to lysate, and mix by pulse-vortexing for 15 sec. After this step, briefly centrifuge the tube to remove drops from the inside of the lid. Transfer the mixture to spin column (fit in a 2 ㎖ collection tube).**

5. **Centrifuge at 13,000 rpm for 30 sec at room temperature. And discard the pass-through and reinsert the spin column into the same collection tube.**

6. **Add 700 µl of Buffer VRW1 to the spin column. Centrifuge at 13,000 rpm f or 30 sec at room temperature. And discard the pass-through and reinsert t he spin column into the new collection tube.**

7. **Add 700 µl of Buffer VRW2 to the Spin column. Centrifuge at 13,000 rpm f or 30 sec at room temperature. And discard the pass-through and reinsert t he spin column into the new collection tube.**

8. **Centrifuge at 13,000 rpm for 1 min at room temperature to remove residual wash buffer. Transfer the spin column to a new collection tube.**
   · Residual ethanol may interfere with downstream reactions.

9. **Add 50~150 µl of Buffer VRE to the center of the membrane in spin Col umn, and wait for at least 1 min at room temperature.**

10. **Centrifuge at 13,000 rpm for 1 min at room temperature.**
    · Purified RNA/DNA can be stored at -20 ℃ for immediate use and stored at -70 ℃ for long term storage.