Purification of viral DNA/RNA using Ribospin™ vRD and Ribospin™ vRD II

These protocols are especially designed for isolation of viral DNA/RNA from whole blood, saliva, urine, stool, tissue, sputum and dried blood spot

Preparation
- Preheat a water bath at 56℃, 85℃
- Proteinase K (20 mg/ml)
- 1.5 ml microcentrifuge tube
- 1X PBS
- 2 ml Glass Bead or 5 mm diameter Stainless Steel Bead
- Buffer CL

1 Whole Blood, Urine

1. Dilute whole blood to 1:5 volume ratio with 1X PBS.
2. Continue with step 1 of each protocol.

2 Stool

* Good performance can be achieved without pre-treatment, but pre-treatment described below might be an option to enhance extraction efficiency.

1. Add 50~100 mg of stool or 50~100 µl of diarrhea stool to 2 ml Glass Bead tube or 5 mm diameter Stainless Steel Bead tube.
2. Add 400 µl of Buffer VL to the tube. (Ribospin™ vRD II : Buffer NVL)
   Buffer VL of Ribospin™ vRD or Buffer NVL of Ribospin vRD™ II can be replaced with PBS.
3. Vortex for more than 1 min.
4. Incubate for 1 min at room temperature.
5. Transfer 200 µl of supernatant to new tube without disturbing the precipitate.
6. Continue with step 1 of each protocol.

3 Tissue

* TissueLyser II method

1. Add 50~100 mg of tissue sample to 2 ml tube with 6 mm diameter Stainless Steel Bead.
2. Add 400 µl of Buffer VL to the tube. (Ribospin™ vRD II : Buffer NVL)
3. Grind the tissue at 30 Hz for 30 sec using TissueLyser II.
4. Add 400 µl of Buffer VL. (Ribospin™ vRD II : Buffer NVL)
5. Vortex for 30 sec and incubate for 1 min at room temperature.
6. Transfer 200 µl of supernatant to new tube without disturbing the precipitate.
7. Continue with step 1 of each protocol.

* Pestle method

1. Grind 100 mg of tissue with Pestle for less than 1 min.
2. Add 400 µl of Buffer VL. (Ribospin™ vRD II : Buffer NVL)
3. Vortex for 30 sec and centrifuge 13,000 rpm for 1 min at room temperature.
4. Transfer 200 µl of supernatant to new tube without disturbing the precipitate.
5. Continue with step 1 of each protocol.

4 Sputum, Saliva

1. Dilute the sputum to 1:1 volume ratio with PBS.
2. Vortex for 1 or 5 min depending on the sputum viscosity.
3. Continue with step 1 of each protocol.

5 Dried blood spot (FTA card)

1. Place 1~3 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 300 µl of Buffer VL. (Ribospin™ vRD II : Buffer NVL)
2. Incubate at 85℃ for 10 min.
   (Optional) Add 20 µl of Proteinase K solution (20 mg/ml, not provided) and 20 µl of 1M DTT, vortex to mix, and incubate at 56℃ for 10 min.
3. Centrifuge 13,000 rpm for 1 min at room temperature.
4. Transfer 200 µl of supernatant to new tube without disturbing the precipitate.
5. Add 300 µl of Buffer RB1 and gently mix or inverting. Incubate at room temperature for 1 min.
6. Transfer all of the mixture, 500 µl to a Column Type V (mini). [Ribospin™ vRD II : Column Type S (micro)]
7. Continue with step 6 of Ribospin™ vRD protocol. (Ribospin™ vRD II : continue with step 7)

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