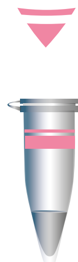
SMARTPURE **Protocol**

Virus RNA

SK-VIPU-100

Please work in an RNase free area and use RNase free tubes and buffers

Sample Preparation



I. For animal tissues (≤30mg) • cultured cells (≤1×10^8) • white blood cells (≤5ml whole blood)

a) Animal Tissues

1. Homogenize the tissue under liquid nitrogen or ice bath and transfer up to 30 mg to a 1.5 mL tube.
2. Add 175 µL of **Lysis Buffer** and mix well.

b) Cultured cells

1. (Detach cells by trypsination) then centrifuge at 1 500 × g for 5 min and discard the supernatant.
2. Add 175 µL of **Lysis Buffer** and mix well.

c) White blood cells

1. Collect white blood cells from whole blood by using Red Blood Cell Lysis Buffer (not included).
2. Add 175 µL of **Lysis Buffer** and mix well.
3. Add 350 µL of **RD Buffer**, mix well and incubate at 70°C for 4 min. Please increase incubation time for hard sample.
4. Centrifuge at 12 000 × g for 10 min and transfer the supernatant into a clean tube. Add 200 µL of ethanol and mix well.

II. For cultured cells (≤1×10^3) • white blood cells (≤ 0.3ml whole blood) • whole blood • plasma • serum...

a) Cultured cells

1. (Detach cells by trypsination) then centrifuge at 1 500 × g for 5 min and discard the supernatant.
2. Add 300 µL of **Lysis Buffer** and mix well.

b) White blood cells

1. Collect white blood cells from whole blood by using Red Blood Cell Lysis Buffer (not included).
2. Add 300 µL of **Lysis Buffer** and mix well.

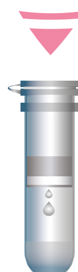
c) Whole blood, plasma, serum

1. Transfer 100 µL of sample

→ If the sample volume ≤100 µL add PBS to 100 µL.

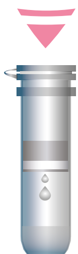
2. Add 300 µL of **Lysis Buffer** and mix well.
3. Add 400 µL of ethanol and mix well.

RNA Binding



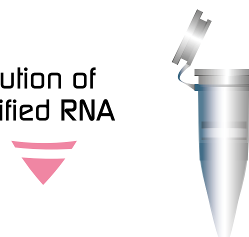
1. Place a SmartPure column into a collection tube (2 mL). Apply the mixture to the SmartPure column and centrifuge at 10 000 × g for 60 sec.
2. Add 200 µL of **DNase Stop Buffer** to the SmartPure column, centrifuge at 12 000 × g for 30 sec and discard the flow-through.

Washing



3. Add 600 µL of **Wash Buffer** to the SmartPure column, centrifuge at 12 000 × g for 30 sec and discard the flow-through.
4. Add 250 µL of **Wash Buffer** to the SmartPure column, centrifuge at 12 000 × g for 120 sec and discard the flow-through.

Elution of purified RNA



5. Transfer the SmartPure column to an RNase free clean 1.5 mL microcentrifuge tube.
6. Add 50 µL of **Elution Buffer** (or RNase-free water pH>7.0) to the SmartPure column and incubate 1 min at room temperature.
7. Centrifuge 1 min at 12 000 × g to recover the total RNA in the microcentrifuge tube.

→ The RNA can be used directly or stored at -80°C for further usage.

The SmartPure Virus RNA kit ensures an easy, fast and effective purification of Virus RNA from various sample types.

FIRST USE

- Add required ethanol to the Wash and DNase Stop buffers (see labels on the bottles) and mix well. RD Buffer may be precipitated at low temperature. Incubate at 37°C for a few minutes to clarify the solution.

Kit components (100 preps)

Component	Amount
SmartPure Lysis Buffer	30 mL
SmartPure RD Buffer	35 mL
SmartPure DNase Stop Buffer	9.8 mL
SmartPure Wash Buffer	40 mL
SmartPure Elution Buffer	20 mL
SmartPure Columns	100

Material not supplied

- → Sterile 1.5 mL microcentrifuge tubes
- → 10/100/1000 µL tips
- → Microcentrifuge (14 000 x g)
- → Water bath
- → Vortex mixer
- → Trypsin
- → Absolute ethanol
- → ß-mercaptoethanol
- → Red Blood cell Lysis Buffer

Shipping & Storage conditions

The SmartPure Virus RNA Kit is shipped at room temperature. The kit is stable for 24 months if stored at room temperature (15-25°C).

Troubleshooting

Low RNA recovery

- RNA may be impaired in rich oxidase content samples therefore reducing recovery yield. Adding 20 µL of ß-mercaptoethanol per mL of Lysis buffer should prevent RNA alteration. Lysis buffer containing ß-mercaptoethanol must be stored at 4°C.
- Please wear gloves, use RNase free clean tips and tubes and work in an RNase free area to avoid any RNase contamination.
- Please store RNA at -80°C and avoid long term storage

RNA Analysis

- RNA concentration is calculated from the absorbance value at 260 nm. To assure significance, absorbance readings at 260 nm must be between 0.1 and 1.0.
- An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. This relation is only valid for measurements in water. Therefore, any dilution of RNA sample should be done in water. Please use RNase-free cuvettes.
- Concentration of RNA sample = 40 µg/ml x OD260 x dilution factor.
  Pure RNA has a OD260/OD280 ratio of 1.9–2.1 in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution. If there is contamination with protein or phenol, the OD260/OD280 ratio will be significantly reduced.

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