Single Cell miTotal RNA Extraction Miniprep Kit

Parts SCRNA-1, SCRNA-2

Product Contents

Cat. No	SCRNA-1	SCRNA-2
Preps	50	250
VRX Buffer	13ml	65ml
WS Buffer (RNA)	12ml	30ml x 2
RNase-free ddH2O	1.5 ml x 2	15ml
RNA Mini Column	50	250
Collection Tube	50	250

Protocol

Important Notes:

Please read the following notes before starting the procedures.

- Add 48ml/bottle (for SCRNA-1) or 120ml/bottle (for SCRNA-2) of 98~100% ethanol into WS Buffer bottle when first opened.
- The bottle of VRX buffer may turn to yellow/brown color overtime, the color changes does not affect RNA purification.
- All plastic wares and containers should be treated properly to make sure they are RNase-free. Gloves should be worn when handling RNA.
- Buffers provided in this system contain irritants. Appropriate safety gear such as gloves and lab coat should be worn.
- All centrifugation steps, except when pelleting cells, should be at full speed (10,000 x g or 13,000 ~14,000 rpm) in a microcentrifuge.
- All procedures should be done at room temperature (20~25°C).

Buffer Preparation

Add 48ml/bottle (for SCVTR1001) or 120ml/bottle (for SCVTR1002) of 98~100% ethanol into WS Buffer bottle.

Sample Preparation

Monolayer cells, aggregated cells, stem cells

Trypsinization of monolayer cells, aggregated cells, or stem cells into single cell suspension is necessary prior to extraction.

Suspension cells

Single cell preparation by serial dilution: It is recommended to use PBS or chemically defined media to dilute the cells or treat the cells before RNA extraction.

Confirmation of single cells

After serial dilution, it is important to confirm that only a single cell is in the final dilution. if more than one cell is in the final dilution, the downstream quantitative measurement will not be accurate for a single cell.

1. **To ensure that the final dilution contains only a single cell, visualize 1/10 volume of the final dilution under a microscope and repeat 9 times.** You should only observe a single cell. For example, if the final dilution is 100µl, pipet 10µl aliquots onto a counting chamber, such as hemocytometer, and observe under a microscope.

2. **Once the presence of a single cell is confirmed, split the final dilution into two equal volumes into 1.5ml tubes (e.g. if the final dilution is 100µl, split into 50µl), one tube will contain the single cell and the other is the blank control tube.** At this point, drugs, hormones, growth stimulants or inhibitors, or environmental factors (e.g. temperature, oxygen levels etc) can be used for treatment of the cell.

3. **Add three times the volume of VRX buffer to the one volume of the sample.** For example, for a 60µl sample, 180µl VRX buffer is needed. Vortex the sample to lyse the cell.

4. **Add one volume isopropanol (98~100% not provided) directly to one volume sample homogenate (1:1) in VRX buffer**. For example 200µl add 200µl isopropanol. Mix well by vortexing.

5. **Load the mixture into a miTotalTM RNA Column in a collection tube and centrifuge for 1 minute. Transfer the column into a new collection tube and discard the collection tube containing the flow-through.**

6. **Wash the column/ collection tube twice with 500µl WS buffer (ethanol added) by centrifugation for 1 minute. Discard the flow-through.** For complete removal of the residual wash buffer, centrifuge the column for an additional 5 minutes, as residual ethanol may inhibit reverse transcriptase activity. Transfer the column carefully into an RNase-free tube (not provided).

7. **Add 25µl of RNase-Free water directly to the column matrix and incubate for 1min, then centrifuge at max speed for 1 minute to elute the RNA.** The eluted RNA can be used immediately or stored at -70 °C.

Troubleshooting Observation	**Possible cause**	**Comments/suggestions**
No detectable RNA by RT PCR, RT qPCR	Incorrect cell counting or RNase contamination	Visualization of cells under microscope. Keep samples frozen until RNA extraction. Whenever possible, fresh samples should be used and processed immediately. Store samples at -20°C or below -70°C/liquid nitrogen or RNA Stabilization reagent immediately after harvesting cells. Freeze & thaw cycles should be avoided. Wear RNase-free gloves during all procedures. Use only sterilized and RNase-free glass and plastic wares.
No enzymatic reaction	Ethanol residue	Ensure that the ethanol is completely removed prior to the elution step.