User Bulletin

VIOGENE
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96 wells Single cell miTotal RNA Extraction Miniprep System

Viogene **96 wells *Single cell miTotal*** RNA Extraction Miniprep System provides a fast method to purify total RNA of a single cell from various samples such as cells from culture or blood buffy coat. A simple spin-column based method is used to isolate large RNAs, siRNAs and microRNAs without the time-consuming procedure of phenol/chloroform extraction and ethanol precipitation, the highly purified total RNA can be used for quantitative detection of RNA level using quantitative method, such as qPCR, digital PCR or microarray.

Sample Preparation Time: ~ 30 minutes, operation under biosafety hood.

For Research Use Only

Downstream Application

- qPCR
- Digital PCR
- Microarray
- cDNA synthesis
- RT-PCR
- NGS
- Gene editing

Product Contents

Cat. No	**SCVTR961001**
Preps	96 x 4
VRX Buffer	110ml
WS Buffer (RNA)	2x50ml
RNase-free ddH_2O	25 ml
SCVTR96 Column Plate	4
SCVTR96 Collection Plate	4
Protocol	1

Shipping & Storage

Viogene **96 wells Single cell miTotal** RNA Extraction Miniprep System is stable at 20-25°C for one year. The kit should be stored in a dry place and kept away from direct sunlight.

Must-read Notes:

Please read the following notes before starting the procedures.

- Add 200 ml/bt of 98-100% ethanol into WS Buffer bottle when first open.
- 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 should be done at full speed of a swing-bucket centrifuge (3,000-5000 x g or 4,000-6000 rpm).**
- All procedures should be done at room temperature (20-25°C).

Protocol:

Buffer Preparation

Add 200 ml/bt 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.

Suspension Cells

Single cell preparation by serial dilution: It is recommended to use PBS or chemically defined media (such as serum free) 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 1.5 ml 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 each sample of the mixture into **each RNA Mini Column of the SCVTR96 Column Plate with (sitting on) a SCVTR96 Collection Plate,** then centrifuge for at 3,000-5000 x g for 5 minutes. Discard the waste of collection plate containing the flow-through. In order to balance the centrifugation, the samples should either split as a duplex, or using blank samples for the balancing.

6. Wash the column/collection plate twice with **400µl WS buffer** (ethanol added) by centrifugation at 3,000-5000 x g for 5 minutes. Discard the flow-through. For complete removal of the residual washing buffer, centrifuge the column/collection plate for an **additional 5 minutes**, as residual ethanol may inhibit reverse transcriptase activity. Transfer the column plate carefully into an RNase-free 96 well micro-plate (not provided).

7. Add 25-50µl of RNase-Free water **directly to each column of the column/plate** and centrifuge at of 3,000-5000 x g for **10 minutes** 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.