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VIOGENE®

Ver. : 01A

User Bulletin

Single cell miTotal™ RNA Extraction Miniprep System

Viogene® ***Single cell miTotal***™ RNA Extraction Miniprep System provides a fast method to purify total RNA from 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

Product Contents

Cat. No	SCVTR1001	SCVTR1002
Preps	50	250
VRX Buffer	13ml	65ml
WS Buffer (RNA)	12ml	30ml x 2
RNase-free ddH_2O	1.5 ml x 2	15ml
RNA Mini Column	50	250
Collection Tube	50	250
Protocol	1	1

Shipping & Storage

Viogene ***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.

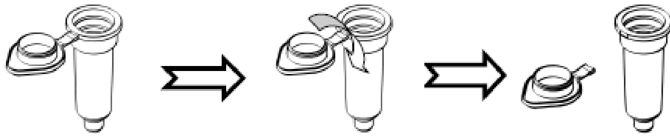
- Add 48 ml/bt (for SCVTR1001) or 120 ml/bt (for SCVTR1002) of 98-100% ethanol into WS Buffer bottle when first open.
- The bottle of VRX buffer may turn to yellow/brown color overtime, the color changes does not affect RNA purification.
- All plastic wares and containers should be treated properly to 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, 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).

Viogene's unique design — EasyLid™

The EasyLid™ is designed to prevent contamination during the procedure.

Tips for EasyLid™ –

Twist the arm of the cap and pull the cap to break the EasyLid™.



Protocol:

Buffer Preparation

Add 48 ml/bt (for SCVTR1001) or 120 ml/bt (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.

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 100 μl, pipet 10 μl 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 100 μl, split into 50 μl), 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 **RNA Mini 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 **500 μl WS buffer** (ethanol added) by centrifugation for 30-60 seconds. Discard the flow-through. For complete removal of the residual washing 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 25 μl of RNase-Free water **directly to the column matrix** and 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.

Serial Products

Product Name	Cat. No.	Preps
miTotal™ RNA Miniprep	VTR1001	50
	VTR1002	250
VioTotal™ Plant RNA Miniprep	PVTR1001	50
	PVTR1002	250

Different size of Viogene ***miTotal***™ and ***VioTotal***™ RNA Extraction Systems.