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User Bulletin

miTotal™ RNA Extraction Miniprep System

Enhanced microRNAs Purification

Viogene® ***miTotal***™ RNA Extraction Miniprep System provides a fast method to purify total RNA from various samples such as cells from culture, tissues, whole blood, plasma, serum, biological fluids containing RNA virus, *etc*.. A simple spin-column based method can isolate large RNAs, siRNAs, **microRNAs**, and viral RNAs without the time-consuming procedure of phenol/chloroform extraction and ethanol precipitation.

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

For Research Use Only

Downstream Application

* Northern blotting
* Ploy A^+ RNA selection
* cDNA synthesis
* RT-PCR
* Transcription profiling

Product Contents

Cat. No	VTR1001	VTR1002
Preps	50	250
VRX Buffer	26ml	130ml
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 ***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 VTR1001) or 120 ml/bt (for VTR1002) of 98-100% ethanol into WS Buffer bottle when first open.
- All plastic wares and containers should be treated properly to make sure RNase-free. Gloves should be worn when handling RNA.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- All centrifugation steps except cell pelleting, should be at full speed (10,000 *x g* or 13,000 - 14,000 rpm) in a microcentrifuge.
- All procedure 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 VTR1001) or 120 ml/bt (for VTR1002) of 98-100% ethanol into WS Buffer bottle when first open.

Sample Preparation

Various guidelines are provided for monolayer cells, suspension cells, biological fluids (whole blood, plasma, serum, buffy coat, CSF, semen, saliva, and body fluids), and tissues.

Monolayer Cells

It is recommended to process $1x10^4$ – $5x10^6$ animal cells (per prep).

1. To lyse cells, add 100 μl VRX per cm^2 dish/plate (see table below) directly on the surface area of the adherent monolayer cell culture, mix well by pipetting, transfer the mixture into a RNase-free microcentrifuge tube, and vortex to completely lyse the cells.
   Example: Add 400 μl VRX per well of a 12-well plate (table below).

2. Centrifuge the mixture at 10,000 *x g* for 2 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube (not provided). Proceed with RNA Purification.

Approximate confluent cell number per culture area.

Culture Container	Well/Flask Surface	Cell Number
6-well plate	9-10 cm^2	$0.5\text{-}1x10^6$
12-well plate	4 cm^2	$4\text{-}5x10^5$
24-well plate	2 cm^2	$1\text{-}3x10^5$
96-well plate	0.3-0.6 cm^2	$4\text{-}5x10^4$
T25 Culture Flask	25 cm^2	$2\text{-}3x10^6$
T75 Culture Flask	75 cm^2	$0.5\text{-}1x10^7$
T175 Culture Flask	175 cm^2	$2\text{-}3x10^7$

Suspension Cells

It is recommended to process $1x10^4$ – $5x10^6$ animal cells (per prep).

1. Spin down cells by centrifugation (up to $5x10^6$ animal cells). Carefully remove the supernatant and resuspend cells in 100 μl (one volume) of PBS, add 300 μl (three volumes) of VRX buffer to lyse the cell by vortexing.
   Note: After adding VRX buffer, the samples is stable for later step of total RNA purification in room temperature.

2. Centrifuge the mixture at 10,000 *x g* for 2 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube (not provided). Proceed with RNA Purification.

Biological Fluids

100 μl of biological liquid per prep (whole blood, plasma, serum, buffy coat, CSF, semen, saliva, biological fluids containing RNA virus and body fluids) can be processed.

1. For 100 μl (one volume) of biological liquid, add 300 μl (three volumes) of VRX buffer to mix the biological liquid by vortexing.

2. Centrifuge the mixture at 10,000 *x g* for 2 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube (not provided). Proceed with RNA Purification.
   Note: When transfer supernatant of **whole blood** or **plasma,** avoiding bottom red blood phase

Tissue

Up to 50 mg of tissue sample can be used in each prep.

1. To complete homogenize 50 mg tissue sample, add 500 μl VRX buffer into the sample container, disrupt and homogenize with a homogenizer (20-G needle, Polytron, *etc*.).
   Note: If samples already stored in VRX buffer, adjust the ratio to 1:10 (sample: VRX), sample should not exceed 10% of the VRX volume.

2. Centrifuge the mixture at 10,000 *x g* for 2 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube (not provided). Proceed with RNA Purification.

RNA Purification

1. Add **one volume isopropanol** (98-100% not provided) directly to one volume sample homogenate (1:1) in VRX buffer. Mix well by vortexing.

2. Load the mixture into a ***miTotal***™ **RNA Column** in a collection tube and centrifuge for 1 minute. Discard the flow-through.

3. **Wash two times** of the column/collection tube with **500 μl WS buffer** (ethanol added) by centrifugation for 30-60 seconds. Discard the flow-through. To complete removal of the residuals washing buffer, centrifuge the column for an **additional 5 minutes**, as residual ethanol may cause the low A_{260}/A_{230} and inhibit reverse transcriptase activity. Transfer the column carefully into an RNase-free tube (not provided).
   Note: At this point, RNA samples can be in-column DNase treated optionally, by adding 50 μl of DNase I cocktail (5 U DNase I, 5 μl 10X DNase I reaction buffer, and 45 μl RNase free water), incubate at 25-37°C for 10 minutes, after the incubation, add 100 μl VRX/ 100 μl isopropanol (total 200 μl)mix into the column, centrifuge for 1 minute, discard the flow-through, then process to Step 3 and 4 of the RNA Purification.

4. Add 50 μl of RNase-Free water **directly to the column matrix** and centrifuge at max speed for 1 minute.
   The eluted RNA can be used immediately or stored at -70 °C.

Troubleshooting

Observation	Possible cause	Comments/suggestions
Low or no RNA yield	Inefficient lysis of sample	Make sure that completes homogenization and disruption of samples. Use sufficient starting materials as suggested. Decrease it, if more than suggested amount of the sample used.
	In sufficient sample storage	Keep samples freezed until RNA extraction, avoiding samples freeze and thaw. If problem persist, use fresh samples and process immediately. Storage samples at -20˚C or colder - 70˚C/liquid nitrogen.
	Column clogging	Too much starting materials used. Decrease the samples to suggested sample amount. Incomplete sample and VRX sample mixing. Try longer vortexing time of the mixture. Check lysate for any tissues or particle remaining. Remove particles by centrifuge for 5 minutes/ 10,000xg and transfer supernatant to a new tube. Make sure that completes homogenization and disruption of samples. Use sufficient starting materials as suggested. Decrease it, if more than suggested amount of the sample used Centrifugation temperature too low. The centrifugation temperature should be 20–25˚C.
Degraded RNA, smear	RNase contamination	Samples stored or handled incorrectly: Keep samples freezed until RNA extraction. Whenever possible, fresh samples should be used and processed immediately. Storage samples at -20˚C or colder - 70˚C/liquid nitrogen or RNA Stabilization reagent immediately after cells harvesting. Freeze & thaw cycles should be avoided. Ware RNase-free gloves during all procedures. Use only sterilized and RNase-free glasses and plasticwares.
DNA contamination		Apply DNase I treatment of the suggested step.
No enzymatic reaction	Residues of ethanol	Before RNase-free water elution step, ensure the additional 3 minutes spin step to eliminate the residues ethanol from the washing 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.