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VIOGENE
Ver. : 12A

User Bulletin

96 wells *miTotal*™ RNA Extraction Miniprep System

Enhanced microRNAs Purification

Viogene® 96 wells ***miTotal***™ RNA Extraction Miniprep System provides a high throughput 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 96 wells 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.

Downstream Application

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

Product Contents

Cat. No	VTR96 1001
Preps	96 x 4
VRX Buffer	130ml
WS Buffer (RNA)	2 x 45ml/bt
RNase-free ddH_2O	25ml
VTR96 column plate	4
VTR96 Collection plate	8
Protocol	1

Shipping & Storage

Viogene 96 wells ***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 180 ml/bt of 98-100% ethanol into each WS Buffer bottle (two bottles per kit) 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 should be done at full speed of a swing-bucket centrifuge (3,000-5000 *x g* or 4,000-6000 rpm).**
- All procedure should be done at room temperature (20-25˚C).

Protocol:

Buffer Preparation

Add 180 ml/bt of 98-100% ethanol into each WS Buffer bottle when first open.

Sample Preparation

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

Approximate confluent cell number per culture area.

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

Monolayer Cells

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

1. To lyse cells, add 100 µl of VRX buffer per cm^2 dish/plate (see table above) directly on the surface area of the adherent monolayer cell culture, mix well by pipetting, transfer the mixture into a RNase-free microcentrifuge tube or 96 plate (if use 96-well plate), and completely lyse the cells by vortexing (for tube sample) or pipetting (for 96-well plate sample).
   Example: We suggest adding 100 µl VRX per well of a 96-well plate (table above).

2. Centrifuge the mixture at 3,000-5000 *x g* for 10 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube or collection plate. Proceed with RNA Purification.

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 (for 96 wells plate sample) of VRX buffer to lyse the cell by vortexing (for tube sample) or pipetting up and down (for 96 wells plate sample).

2. Centrifuge the mixture at 3,000-5000 *x g* for 10 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube or collection plate. 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 3,000-5000 *x g* for 10 minutes to remove particulates, and then transfer the supernatant into an RNase-free microcentrifuge tube or collection plate. Proceed with RNA Purification.

   Note: When transfer supernatant of **whole blood** or **plasma,** avoiding bottom red blood phase

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 or pipetting.

2. Set 96-well miTiotal RNA column plate on a collection plate.

3. Transfer the mixture into the wells of **96-well *miTotal*™ RNA column plate** and centrifuge at 3,000-5000 *x g* for 5 minutes. Discard the flow-through in the collection plate.
   For biological fluids, repeat loading 400 μl of the mixture into the wells twice.

4. **Wash** the 96 well column/collection plate **twice** with **400 μl of WS buffer** (ethanol added) by centrifugation at 3,000-5000 *x g* for 5 minutes. Discard the flow-through. To complete removal of the residuals washing buffer, centrifuge the column for an **additional 10 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 elution plate (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.

5. Add 50 μl of RNase-Free water **directly to the column matrix** and centrifuge at max speed for 5 minutes.
   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	Ensure that complete 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. Ensure that complete 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.