User Bulletin

VIOGENE
www.viogene.com

Ver.17A

VioTotal Plant RNA Extraction Miniprep System

Viogene ***VioTotal*** Plant RNA Extraction Miniprep System provides a fast method to purify total RNA from 100 mg plant material or 1 x 10^7 plant cells. A simple spin-column based method can isolate large RNAs, siRNAs, and **microRNAs** 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
- NGS
- Gene editing

Product Contents

Cat. No	PVTR1001	PVTR1002
Preps	50	250
VRX Buffer	26ml	130ml
WS Buffer (RNA)	12ml	30ml x 2
RNase-free ddH_2O	1.5ml x 2	15ml
RNA Mini Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene ***VioTotal*** Plant 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 PVTR1001) or 120 ml/bt (for PVTR1002) 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.

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

Protocol:

Buffer Preparation

Add 48 ml/bt (for PVTR1001) or 120 ml/bt (for PVTR1002) of 98-100% ethanol into WS Buffer bottle when first open.

Sample Preparation

Various guidelines are provided for plant materials and suspension plant cells.

Plant Material

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

1. Grind 50 mg plant material sample **under liquid nitrogen** to a fine powder and transfer to a new tube.

2. 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.

3. 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.

Suspension Cells

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

1. Spin down cells by centrifugation (up to $5x10^6$ plant cells). Carefully remove the supernatant and resuspend cells in 100µl (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.

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 ***VioTotal* 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.

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 3 minutes**. 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^{o}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 $^\circ$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 / 10,000 *x g* 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.