Plant Virus RNA Kit

For research use only

Catalogue Numbers	Quantity
PVR050	50 rxns
PVR100	100 rxns

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Plant Virus RNA Kit uses a simple and efficient spin column procedure to purify virus RNA from a wide range of virus infected plant species including those with high levels of polysaccharide inhibitors. In the presence of a binding buffer and chaotropic salt, total RNA in the lysate binds to the glass fiber matrix of the spin column. The procedure does not require phenol extraction or alcohol precipitation and can be completed within 25 minutes. A variety of high-quality plant viral RNA can be purified from 100 mg plant tissue samples and can be used directly in a variety of sensitive downstream applications.

Quality Control

The Plant Virus RNA Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Virus RNA is purified from leaf discs of grapevine plants infected with multiple viruses. Following RNA purification a 5 µl aliquot from a 50 µl eluate of purified viral RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Advantages

- Purify high-quality virus RNA from a variety of plant species (including those with high levels of polysaccharide inhibitors)
- Time: within 25 minutes
- Includes individually packaged spin columns and collection tubes (certified DNase and RNase-free)
- Elution volume: 50 µl

Applications

RT-PCR, RT-qPCR, Next Generation Sequencing (NGS), Northern Blotting

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

Additional Requirements

absolute ethanol, 95-100% ethanol, ß-mercaptoethanol, 1.5 ml microcentrifuge tubes (RNase-free)

Components and Storage

Item	Volume	Product	Shipping	Storage
PVR Buffer	4 ml	PVR004	room temperature	dry at room temperature (15-25ºC)
	55 ml	PVR050		
	110 ml	PVR100		
PVRS Buffer	400 µl	PVR004	room temperature	dry at room temperature (15-25ºC)
	6 ml	PVR050		
	12 ml	PVR100		
W1 Buffer	2 ml	PVR004	room temperature	dry at room temperature (15-25ºC)
	30 ml	PVR050		
	50 ml	PVR100		
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	PVR004	room temperature	dry at room temperature (15-25ºC)
	12.5 ml (50 ml)	PVR050		
	25 ml (100 ml)	PVR100		
RNase-free Water	1 ml	PVR004	room temperature	dry at room temperature (15-25ºC)
	6 ml	PVR050		
	15 ml	PVR100		
PV Columns	4 pcs	PVR004	room temperature	dry at room temperature (15-25ºC)
	50 pcs	PVR050		
	100 pcs	PVR100		
2 ml Collection Tubes	8 pcs	PVR004	room temperature	dry at room temperature (15-25ºC)
	100 pcs	PVR050		
	200 pcs	PVR100		

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use

RNA Purification Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

DNA Removal Options: For DNA-free RNA perform either option 1 (following RNA Binding) or option 2 (following RNA Elution).

1. Sample Preparation (please choose 1 of the following options)

a. Cut off **up to 100 mg of fresh or frozen plant tissue**. Freeze the sample with liquid nitrogen. Grind the sample to a fine powder then transfer to a 1.5 ml or 2 ml microcentrifuge tube. Add **1 ml of PVR Buffer, 100 µl of PVRS Buffer and 10 µl of ß-mercaptoethanol** then incubate at 70ºC for 10 minutes. During incubation, mix the sample every 3 minutes or use a Thermomixer. Following incubation, centrifuge at 14-16,000 x g for 5 minutes. Proceed directly to step 2 RNA Binding.

b. Transfer **up to 100 mg of fresh or frozen plant tissue** to a beadbeater-type instrument. Add **1 ml of PVR Buffer, 100 µl of PVRS Buffer and 10 µl of ß-mercaptoethanol**. Homogenize the sample completely then transfer to a 1.5 ml or 2 ml microcentrifuge tube and incubate at 70ºC for 10 minutes. During incubation, mix the sample every 3 minutes or use a Thermomixer. Following incubation, centrifuge at 14-16,000 x g for 5 minutes. Proceed directly to step 2 RNA Binding.

c. Transfer **up to 100 mg of fresh or frozen plant tissue** to a homogenization bag. Add **2 ml of PVR Buffer and 20 µl of ß-mercaptoethanol**. Homogenize the sample completely then transfer **1 ml of the homogenate** to a 1.5 ml or 2 ml microcentrifuge tube. Add **100 µl of PVRS Buffer** then incubate at 70ºC for 10 minutes. During incubation, mix the sample every 3 minutes or use a Thermomixer. Following incubation, centrifuge at 14-16,000 x g for 5 minutes. Proceed directly to step 2 RNA Binding.

NOTE: If the bottle of PVR Buffer will be used within 1 month, 500 µl of ß-mercaptoethanol may be added to the 55 ml PVR Buffer bottle or 1 ml of ß-mercaptoethanol may be added to the 110 ml PVR Buffer bottle.

2. RNA Binding

1. Transfer **450 µl of supernatant** to a new 1.5 ml or 2 ml centrifuge tube then add **225 µl of 95-100% ethanol**.
2. Place a **PV Column** in a 2 ml Collection Tube then transfer the **ethanol-added mixture to the PV Column**.
3. Centrifuge at 14-16,000 x g for 1 minute. If the mixture could not flow past the PV Column membrane following centrifugation, increase the centrifuge time until it passes completely.
4. Discard the flow-through then place the **PV Column** in a new 2 ml Collection Tube.

DNA Removal Option 1: Add 100 µl of DNase I (2 KU/ml) mixed in a reaction buffer (e.g. 50 mM Tris-HCl pH7.5, 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC or buffer provided with DNase I) to the center of the PV Column matrix. Let stand for 10 minutes at room temperature then proceed to step 3 RNA Wash.

NOTE: For most viral RNA detection processes via RT-PCR, DNA removal is not required.

3. RNA Wash

1. Add **400 µl of W1 Buffer into the center of the PV Column** then centrifuge at 14-16,000 x g for 1 minute.
2. Discard the flow-through then place the **PV Column** back in the 2 ml Collection Tube.
3. Add **600 µl of Wash Buffer (make sure ethanol was added)** to the **CENTER** of the **PV Column**.
4. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through and place the **PV Column** back in the 2 ml Collection Tube.
5. Add **600 µl of Wash Buffer (make sure ethanol was added)** to the **CENTER** of the **PV Column**.
6. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through and place the **PV Column** back in the 2 ml Collection Tube.
7. Centrifuge at 14-16,000 x g for 2 minutes to dry the column matrix.

4. RNA Elution

1. Place the dried **PV Column** in a clean 1.5 ml microcentrifuge tube (RNase-free).
2. Add **50 µl of RNase-free Water** or TE (RNase-free) to the **CENTER** of the column matrix.
3. Let stand for 2 minutes or until the **RNase-free Water** or TE (RNase-free) is absorbed completely by the matrix.
4. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

NOTE: If higher RNA concentration is required, repeat step 4 using the final eluate.

DNA Removal Option 2: Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer (e.g. 50 mM Tris-HCl pH7.5, 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC or buffer provided with DNase I) to the final elution sample. Let stand for 10 minutes at room temperature.

NOTE: For most viral RNA detection processes via RT-PCR, DNA removal is not required.

Plant Virus RNA Kit Real-Time PCR Data

Detection of Grapevine Leaf-Roll Associated Virus 3 (GLRaV-3) – Highly Damaging

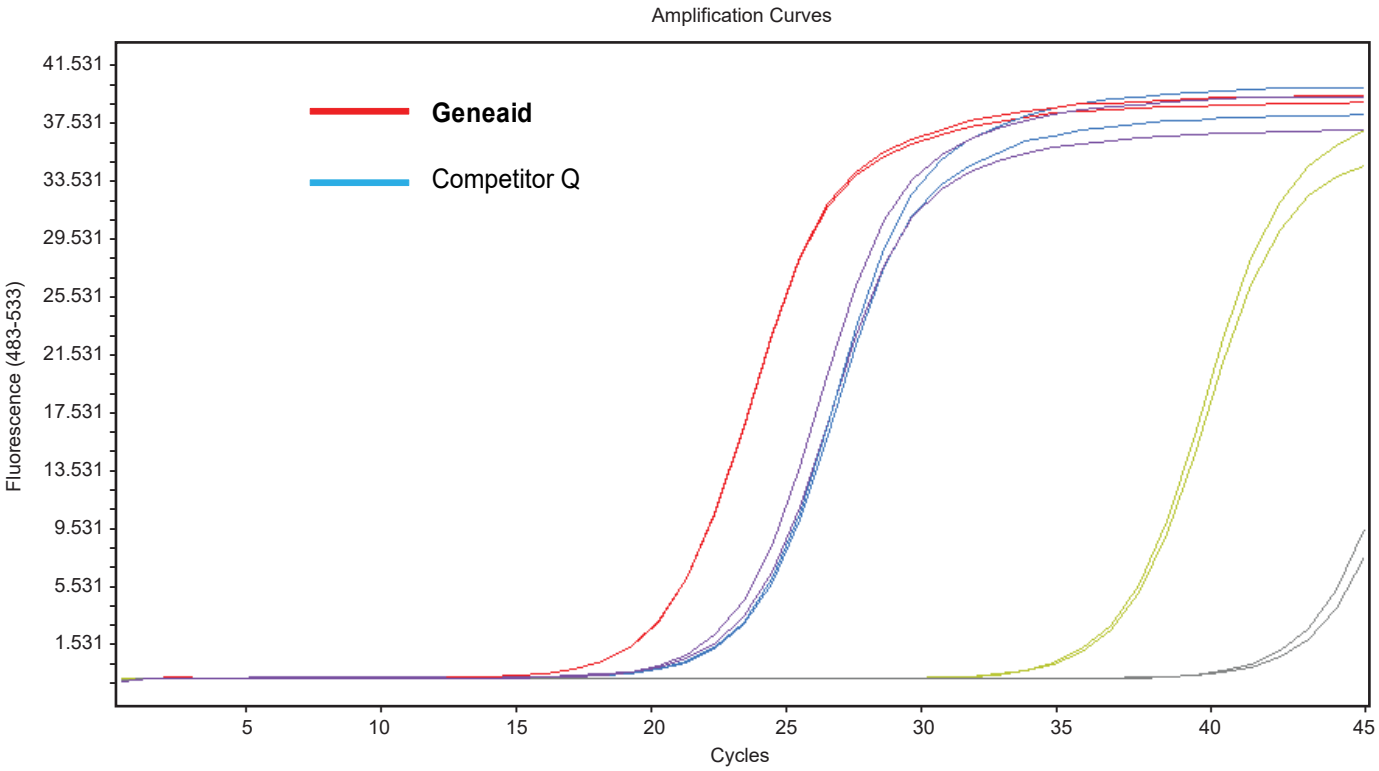

Figure 1. Real-Time PCR data confirms GLRaV-3 was successfully detected and identified following virus RNA extraction using the Geneaid Plant Virus RNA Kit. Increased analytical sensitivity was evident by the earlier (lower) Cq – Cycle quantity – value compared to Competitor Q.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Incorrect RNA elution	A. Make sure RNase-free Water is added to the center of the PV Column and is absorbed completely.
Degraded RNA	A. Incorrect sample storage temperature	A. Extracted RNA should be stored at -70°C.
Low RNA A260/A280	A. Incomplete wash step	A. Wash the PV Column with ethanol added Wash Buffer 2 times.
Eluted RNA does not perform well in downstream applications	A. Residual ethanol contamination	A. Following the wash step, dry the PV Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes.
Sample too viscous	A. Sample not mixed sufficiently during lysis	A. Use a Thermomixer or more frequent mixing of the sample

Related RNA Purification Products

RNA Extraction		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	RBM10/25
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Maxi Kit (Tissue)	10/25 preps	RTM10/25
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Total RNA Maxi Kit (Plant)	10/25 preps	RPM10/25
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
96-Well Total RNA Extraction Kit	4/10 x 96 preps	RBP04/10
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	50/100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 rxns	GZX050/100/200
TriRNA Pure Kit	50/100/200 rxns	TRP050/100/200
RNA Cleanup Kit	50/100 rxns	PR050/100

For additional product information, please visit www.geneaid.com. Thank you!