GenPlax™ PVM Dual-target cPCR Detection Kit

Cat. No. 867-100 (100T)

Storage at -15 °C to -25 °C

Disclaimer

For research use only.

Description

GenPlax™ PVM Dual-target cPCR Detection Kit is a dual-amplification diagnostic tool designed to detect specific genes of (*Carlavirus misolani,* PVM) using the complete PCR (cPCR) method. The limit of detection is 10^3 copies (plasmid DNA).

This kit includes a hot start PCR enzyme, which helps prevent non-specific amplifications caused by mispriming and primer dimer formation during reaction mixture preparation and other pre-cycling steps.

It also contains uracil DNA glycosylase (UDG)*, which helps prevent contamination from previous PCR products.

Additionally, the kit includes a positive control to verify the accurate location of the target amplification products.

Agarose gel electrophoresis is required to visualize the results using this kit.

* UDG is an enzyme that specifically removes uracil residues from DNA.

Components

Cat. No.	867-100
GenPlax™ PVM Dual cPCR 2X Master Mix (UDG, Dye)	1 mL (500 µL x 2)
GenPlax™ PVM Dual-target Primer Mix	500 µL (250 µL x 2)
GenPlax™ PVM Dual-target Positive Control	500 µL (250 µL x 2)
GenPlax™ Nuclease-Free Water	1 mL (500 µL x 2)

Storage Conditions

Stable for 1 year at -15 °C to -25 °C.

Protocol

1. Sample preparation

Prepare the total RNA (or viral RNA) extracted from PVM-infected plants or plants exhibiting PVM-like symptoms. Obtain cDNA products by using 1 µg of the total RNA with cDNA synthesis kit containing reverse transcriptase.

※ Recommendation

RNA purity should reach 2.0 in the A_{260}/A_{280} absorbance ratio. Total RNA can be obtained from plants using the Ribospin™ Plant (GeneAll) or AllEx® Mini Plant Total RNA Kit / AllEx® Mini Plant DNA/RNA Kit / AllEx® Plant Total RNA Kit / AllEx® Plant DNA/RNA Kit (GeneAll).

2. Reaction preparation

1) Thaw all components completely on ice (or in a cooler).
2) Vortex briefly, then spin down to collect all components at the bottom of the tube.
3) Prepare the mixture according to the following table.

Component	Sample	Positive Control
GenPlax™ Nuclease-Free Water		-
GenPlax™ PVM Dual cPCR 2X Master Mix (UDG, Dye)	10 µL	10 µL
GenPlax™ PVM Dual-target Primer Mix	5 µL	5 µL
cDNA products *	Up to 5 µL	-
GenPlax™ PVM Dual-target Positive Control	-	5 µL
Total volume (per reaction)	**20 µL**	**20 µL**

4) Vortex briefly, then spin down to collect all components at the bottom of the PCR tube.

※ Recommendation

All steps should be performed on ice (or in a cooler).

* Please refer to the '3. cDNA synthesis (reverse transcription, RT)' section for additional information.

3. Set up of PCR conditions in a thermal cycler

Step	Temp.	Time	Cycles
UDG activation	50 °C	3 min	1
UDG inactivation / Initial denaturation	95 °C	15 min	1
Denaturation	95 °C	20 sec	40
Annealing	60 °C	20 sec	
Extension	72 °C	30 sec	
Final extension	72 °C	5 min	1

4. **Agarose gel electrophoresis**

   The PCR products can be evaluated by agarose gel electrophoresis followed by DNA staining.

   ※ Recommendation

   Agarose gel can be used at a concentration of 1%, it can be made by dissolving agarose powder in 0.5X TAE buffer. The DNA straining solution can be used DyeAll™ Staining Solution (GeneAll).

   Loading 3 μL – 5 μL of the PCR products onto the agarose gel for electrophoresis.

   The PCR products of the positive control can be loaded as 1 μL onto the agarose gel for electrophoresis.

Analysis of Results

The presence or absence of the target DNA is determined based on the amplicon size. A positive result is indicated by a band appearing at the expected size, identical to the amplicon of the positive control, while a negative result shows no band or a different band size.

PVM	Amplicon Size
Positive	379 bp, 521 bp
Negative	No band/Different band size

Troubleshooting

1. **No band of the positive control amplicon**

   The PCR results have no reliability. Check all components and PCR conditions, then perform the PCR reaction again. Check the expiration date of the used kit. The expiration date is one year.

2. **Target band of the negative control amplicon**

   The PCR results have no reliability. The negative control and PCR components may be contaminated. Check all components and PCR conditions. Remove any contaminated component(s), then perform the PCR reaction again.

Important Information

1. **Non-template control (NTC)**

   The NTC is used to detect contamination in the lab environment. It contains all the components except the template DNA and can be prepared using nuclease-free water. This helps ensure that any amplification is due to the template and not caused by contamination.

2. **Prevention of contamination in the components**

   When preparing the PCR reaction mix, add the sample cDNA and positive control last. Carefully open and close all component tubes.

3. **Use of sterile filter tips and tubes**

   These are used to prevent contamination of the components.

4. **Low amplification efficiency**

   Check the condition of the total RNA and cDNA. When synthesizing cDNA, ensure that the A_{260}/A_{280} ratio of the total RNA is close to 2.0, and confirm that at least 1 μg of total RNA is used.

5. **Recommended storage conditions**

   Kits that are not stored according to the recommended conditions or are past their expiration date may not guarantee performance.

6. **Maintenance of PCR equipment**

   Regularly check the performance of the PCR equipment and sanitize all tools used for PCR.

7. **Prevention of cross-contamination and sample contamination**

   Prepare the PCR reaction mixture in a sanitized area, and discard all used disposable items according to the established disposal protocols, in compliance with the regulations of the respective country.

Precautions for Use

1. This kit is intended for research use only.
2. Repeated freeze-thaw cycles may affect the performance of the kit.
3. Exchanges will not be provided for issues caused by user error or mishandling.
4. The kit should not be used after its expiration date.
5. The procedure must be followed in the specified order, and the kit should be used immediately after opening.

Additional Information

1. Total RNA extraction using the AllEx® Mini Plant Total RNA Kit

The AllEx® Mini Plant Total RNA Kit is a specialized nucleic acid extraction reagent designed for use with the AllEx® Mini Automated Nucleic Acid Extraction System (AllEx® Mini).

a. Sample preparation

1) Grind sample to a fine powder completely using a mortar and pestle under liquid nitrogen.
2) Place up to 100 mg of ground sample into a 1.5 mL Microcentrifuge Tube (not provided).
3) Add 400 µL – 600 µL of Buffer SQ1 to the sample and vortex vigorously for 30 s.
4) Incubate the mixture for 5 min at room temperature. Centrifuge the lysate at 13000 rpm (≥ 10000 × *g*) for 10 min at 4°C.

b. Set up of the cartridge in the AllEx® Mini Plant Total RNA Kit

1) Peel back the seal of the 1st well on the left of the pre-filled reagent cartridge.
2) Transfer the 200 µL of the supernatant from the sample into the 1st well.
3) Dispense 4 µL of the dissolved DNase I solution (provided in the kit) into the 3rd well on the left of the pre-filled reagent cartridge.

c. Insertion of the cartridge into the cassette of the AllEx® Mini

1) Insert up to 12 cartridges into the cassette, starting with position number 1 as indicated.
2) Remove the remaining sealing film.

d. Operation of the AllEx® Mini

1) Open the door of the AllEx® Mini.
2) Load the cassette with the cartridge into the AllEx® Mini, ensuring the diagonally cut edge faces the system door.
3) Tap the play icon to scan the barcode on the cartridge.
4) The built-in barcode reader scans the cartridge barcode, selects the protocol, and displays the protocol list.
5) Select the appropriate protocol from the list based on your uses and purposes.
6) Tap 'Confirm' to start the extraction.

2. Total RNA extraction using the Ribospin™ Plant Kit

Ribospin™ Plant kit is specially designed for purification of total RNA from various plant tissues such as leaves, stems, roots and picky plant samples. This kit provides the optimized buffer and spin column, which is effective at removing polysaccharides and polyphenolic compounds and isolating intact plant RNA.

a. Sample preparation

1) Prepare a plant tissue sample up to 100 mg, then grind the sample into a fine powder using a mortar and pestle with 250 µL of Buffer RPL.
2) Transfer the ground sample into a 1.5 mL Microcentrifuge Tube (not provided).
3) Incubate for 3 min at room temperature.

b. EzPure™ Filter

1) Transfer the lysate to an EzPure™ Filter.
2) Centrifuge at ≥ 10000 × *g* for 30 s at room temperature.
   Through this step, large cell debris and most of the genomic DNAs are filtered onto the EzPure™ Filter, and a small pellet of cell debris will form at the bottom of the collection tube.
3) Transfer the supernatant to a new 1.5 mL Microcentrifuge Tube (provided).

c. Binding

1) Add 1 volume (usually 350 µL) of 70% EtOH to the tube containing the supernatant, and mix well by pipetting or inverting.
2) Apply the mixture to a Column Type W (blue ring).
3) Centrifuge at ≥ 10000 × *g* for 30 s at room temperature.
4) Discard the pass-through and reinsert the mini column into the collection tube.

d. DNase I treatment

1) Add 500 µL of Buffer RBW to the mini column.
2) Centrifuge at ≥ 10000 × *g* for 30 s at room temperature.
3) Discard the pass-through and reinsert the mini column into the collection tube.
4) Apply 70 µL of DNase I reaction mixture to the center of the mini column.
5) Incubate for 10 min at the room temperature.
6) Add 500 µL of Buffer RBW to the mini column and incubate for 2 min.
7) Centrifuge at ≥ 10000 × *g* for 30 s at room temperature.
8) Discard the pass-through and reinsert the mini column into the collection tube.

e. Washing

1) Add 500 µL of Buffer RNW to the mini column.
2) Centrifuge at ≥ 10000 × *g* for 30 s at room temperature.
3) Discard the pass-through and reinsert the mini column into the collection tube.
4) Repeat this step once more.

f. Elution

1) Centrifuge at ≥ 10000 × *g* for an additional 1 min at room temperature to remove any residual wash buffer.
2) Transfer the mini column to a new 1.5 mL Microcentrifuge Tube (provided).
3) Add 50 µL of Nuclease-Free Water to the center of the membrane in the mini column.
   To increase the RNA concentration, reduce the elution volume to 30 µL.
4) Centrifuge at ≥ 10000 × *g* for 1 min at room temperature.
5) The purified total RNA can be stored at 4 °C for immediate analysis and or at -80 °C for long-term storage.

3. cDNA synthesis (reverse transcription, RT)

cDNA (complementary DNA) synthesis is a process used to convert RNA into DNA using reverse transcription.

1) Thaw all components of the cDNA synthesis kit (or RT-Kit) completely on ice (or in a cooler).
2) Prepare the mixture according to the following table.

Component	Volume
Nuclease-Free water	
5X reaction buffer	10 µL
8 mM DTT	1 µL
100 pmol/µL random hexamer primers	0.5 µL
Reverse transcriptase or RTase (200 U/µL)	0.5 µL
RNase inhibitor (40 U/µL, optional)	1 µL
Total RNA	1 µg
Total reaction volume (1 reaction)	**50 µL**

3) Vortex briefly, then spin down to collect all components at the bottom of the PCR tube.

※ Recommendation
All steps should be performed on ice (or in a cooler).
Add 100 µL of Nuclease-Free Water to the lyophilized random hexamer (10 nmol) to achieve a final concentration of 100 pmol/µL.