



### Highlights:

- Ready-coated plate
- Dual sensitivity levels
- Two hour protocol
- Consistent quality
- Higher efficiency

### Contents of Kit:

- Anti-Squash Mosaic Virus antibody-coated strip plate (12 strips of 8 wells each, in frame)
- Squash Mosaic Virus Enzyme Conjugate
- 10X Leaf & Seed Extraction Buffer (makes 500 mL)
- Packet Wash Buffer Salts (makes 1L)
- Substrate
- Stop Solution

### Materials Needed:

- multi-channel pipette (100 µL)
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)
- marking pen (indelible)
- tape or Parafilm®
- timer
- distilled or deionized water for preparing Wash Buffer and for diluting 10X Leaf & Seed Extraction Buffer
- seed grinding machine
- wash bottle, or microtiter plate or strip washer
- orbital plate shaker
- microtiter plate reader or strip reader capable of reading 450 nanometers (nm)

Catalog Number AP 062

## Intended Use

The QualiPlate Kit for Squash Mosaic Virus screens for the presence of Squash Mosaic Comovirus (SqMV) in seed or leaf extracts. In studies on seed lots determined to be SqMV positive by other test methods and by comparison with controls, this kit was able to consistently detect the presence of the virus (using minimum sample sizes of 2,000 seeds and minimum sub-sample sizes of 200 seeds). Because leaf tissue can have a higher expression of virus, an alternate protocol using a Conjugate Dilution step is presented (page 3) that better differentiates virus levels in leaf samples.

## Preparation of Solutions

- **Wash Buffer:** Add the contents of the packet of Wash Buffer Salts (phosphate buffered saline, pH 7.4 – 0.05% Tween 20) to 1 liter of distilled or deionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay. Additional 1L dry packets may be purchased from Sigma Chemicals, Cat#P-3563, or similar recipes may be prepared from salts on site. **Note:** Wash Buffer is also used in the Conjugate Dilution Alternative leaf testing protocol.
- **1X Extraction Buffer:** It is not required to refrigerate the 10X Leaf & Seed Extraction Buffer, but if it has been, precipitates will form. Allow to warm to room temperature, then stir or shake to dissolve precipitates completely before proceeding. To make 1X Extraction Buffer, add the entire 50 mL bottle of 10X Leaf & Seed Extraction Buffer to 450 mL of distilled or deionized water in a suitable container, and mix thoroughly to dissolve any remaining precipitates. Store 1X Extraction Buffer refrigerated when not in use; warm to room temperature prior to assay. Additional 10X or 35X buffer may be purchased from EnviroLogix (Cat#KR160 or Cat#KR186 respectively). See "Notes" section for preparing various volumes of Buffer.

## Sample Preparation

**Seeds:** The sample must be extracted with 1X Extraction Buffer at a ratio of 1:10 (gram of seeds to mL of buffer). For example:

- 2.5 g of seed : 25 mL of Extraction Buffer
- 0.25 g of seed : 2.5 mL of Extraction Buffer

All seeds must be thoroughly ground/cracked in order for the internal tissue to come in contact with the buffer (a Braun Aromatic KSR2 coffee grinder with 15 pulses was used for this protocol). Soak ground seed tissue in Extraction Buffer for 1 hour minimum at 4°C. Use the light, non-particulate extract to run the assay.

**Leaf:** The sample must be extracted with 1X Extraction Buffer at a ratio of 1:10 (gram of leaf tissue to mL of buffer). For example:

- 0.5 g of leaf : 5 mL of Extraction Buffer

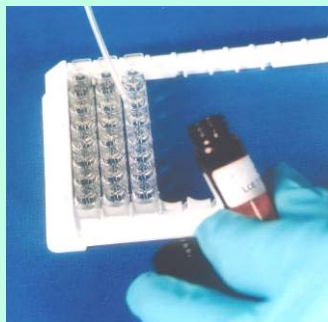
All leaf tissue must be thoroughly macerated in order for ideal sample expression, and may be tested immediately. Centrifuge leaf extract 10 minutes at 5000 x g. Pull off (non-particulated) extract to use for testing.



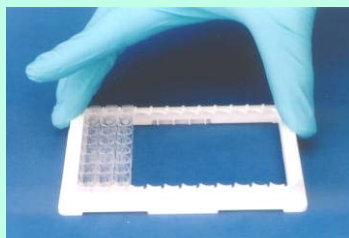
Prepare wash buffer and extraction solutions



Remove unneeded strips



Add Extraction Buffer, controls, and sample extracts



Mix plate, incubate

## How to Run the Assay

- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed plates and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
- **Organize all reagents, sample extracts, and pipettes so that step 1 can be performed in 15 minutes or less;** the use of a multi-channel pipette is strongly recommended for all reagent and extract transfers.
- If more than four strips are to be run at one time, the 15 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see “Note” below).
- If four or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add Blank or sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for each of the three reagents.
- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the foil bag provided, and refrigerate.
- Use the well identification markings on the plate edge as a guide when adding the samples and reagents. It is recommended that at least two wells each of 1X Extraction Buffer as Blanks and known-negative Squash seed or leaf extract be run on each plate. Additional quality control samples may be added at the discretion of the user. Sample extracts may be run in either single or duplicate wells.

### SEED & LEAF PROTOCOL

1. Add **100  $\mu$ L** of **Extraction Buffer (Blank)**, **100  $\mu$ L** of any **user-prepared negative control extract**, and **100  $\mu$ L** of each **sample extract** to their respective wells. Follow the same order of addition for all reagents. Treat each plate as an independently timed assay.

**NOTE:** It is strongly recommended that a multi-channel pipette be used in steps 1, 5, 8 and 9.

2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for a full 20-30 seconds. Be careful not to spill the contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and incubate for **30 minutes** at **ambient temperature** on an **orbital plate shaker at 200 rpm**. Note: Shaking during incubation steps is mandatory where called for. Failure to do so will result in up to 50% loss in assay sensitivity.

*Protocol option: For testing convenience, at this point samples may be incubated overnight in the refrigerator (up to 16 hours at 5°C). Allow plates to come to room temperature with the rest of the kit reagents the next morning, before going on to step 4.*

4. After incubation, carefully remove the covering and empty the contents of the wells into a sink or other suitable container by inverting quickly and vigorously shaking the plate. Flood the wells completely with **Wash Buffer**, then empty as directed above. Repeat this wash step at least three times. After the final wash, keep the plate inverted and tap firmly on a dry paper towel to remove as much Wash Buffer as possible.

*If samples were incubated overnight, increase the number of wash cycles to 8.*



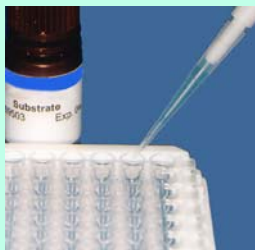
*Bottle Wash method*



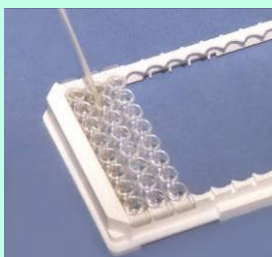
*Strip Plate Wash option*



*Add conjugate, mix, incubate, wash*



*Add substrate, mix, incubate*



*Add Stop Solution*

5. Add **100  $\mu$ L** of **Squash Mosaic Virus Enzyme Conjugate** to each well.
  6. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate** for **1 hour** at **ambient temperature** on an **orbital plate shaker at 200 rpm**. Note: Shaking during incubation steps is mandatory where called for. Failure to do so will result in up to 50% loss in assay sensitivity.
  7. Wash the wells again as described in step 4. Alternatively, perform four washes (300  $\mu$ L/well) with a microtiter plate or strip washer.
  8. Add **100  $\mu$ L** of **Substrate** to each well. Mix thoroughly as in step 2. Cover the wells with new tape or Parafilm and incubate for **30 minutes** (for best results) at **ambient temperature**.
  9. Add **100  $\mu$ L** of **Stop Solution** to each well and mix briefly. This will change the blue color in the wells to yellow. Read the plate at **450 nm**, with a reference wavelength of 600, 630 or 650 nm. Read the stopped plate within 30 minutes; color may fade beyond that time.
- NOTE: Stop Solution is 1 N HCl. Handle carefully.**

#### CONJUGATE DILUTION ALTERNATIVE Leaf Testing Protocol

Because leaf tissue can have a higher expression of virus, it may be difficult to distinguish a relative level of infection. To better differentiate infection levels in leaf tissue only, the Conjugate supplied with the kit may be diluted with Wash Buffer at 1:9.

For example, 2 mL supplied Conjugate : 16 mL Wash Buffer for one full plate.

Perform this dilution just prior to use, making up only as much as needed, and use in Step 5. Discard any excess diluted Conjugate. Additional wash buffer packets may be purchased from Sigma (see page 1).

## How to Interpret the Results

### Spectrophotometric Measurement

Set the wavelength of the microtiter plate reader to **450 nanometers** (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)

### Interpreting Results

Compare the Optical Density (OD) of the sample extracts to those of the mean Extraction Buffer wells, or preferably, to known-negative seed or leaf extract wells, to determine presence or absence of Squash Mosaic Virus in the sample extract. Samples with absorbances significantly greater than those of the Leaf and Seed Extraction Buffer and/or negative extract wells are presumed to be positive for Squash Mosaic Virus.

General Guidelines:

- Mean OD of Extraction Buffer wells should not exceed 0.10.
- Mean OD of SqMV-free squash seed or leaf extracts should not exceed 0.15.

If test results consistently fall outside these guidelines, please contact EnviroLogix' technical service.



Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution.



## Precautions and Notes

- Observe any applicable regulations, federal or state guidelines, or in-house lab safety protocols when disposing of samples and kit reagents.
- Store all QualiPlate components at 4°C to 8°C (39°F to 46°F) when not in use, excluding the 10X Leaf & Seed Extraction Buffer, which should be stored at ambient temperature (18°C to 27°C or 64°F to 81°F).
- Do not expose QualiPlate components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test plates from one QualiPlate with reagents or test plates from a different QualiPlate.
- Do not use samples prepared for analysis in other test kits; do not run sample extracts prepared for this assay in other brands of test kits.
- **Do not expose Substrate to sunlight** during pipetting or while incubating in the test wells.
- **Be sure to read the results of stopped color development at 450 nm, not 405 nm.**
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- Quality of results is dependent upon following the assay protocol as directed.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- **Preparing 1X Extraction Buffer:** The following table shows the formulas for preparing alternative volumes of Buffer. Always make sure the concentrated buffer is in solution before using it.

10X Extraction Buffer (KR160, 50 or 1000 mL)	Finished Volume			
	10L	5L	2L	0.5L
Start with water (L)	9	4.5	1.8	0.45
Add 10X Extraction Buffer (mL)	1000 (1 lg bottle)	500	200	50 (1 sm bottle)

Follow steps in order when diluting 35X Extraction Buffer:

35X Extraction Buffer (KR186, 500 mL)	Finished Volume					
	35L	20L	17.5L	10L	5L	2L
1. Start with water (L)	34	19.43	17	9.71	4.86	1.94
2. Add PVP (g), stir to dissolve	700	400	350	200	100	40
3. Add 35X Extraction Buffer (mL)	1000	571	500 (1 bottle)	286	143	57



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