



### Highlights:

- Ready-coated plate
- Two hour protocol
- Higher efficiency
- Desensitized leaf protocol option

### Contents of Kit:

- Anti-Squash Mosaic Virus antibody-coated solid plate
- Squash Mosaic Virus Enzyme Conjugate
- 1X Leaf Extraction Buffer (50 mL; additional may be purchased from EnviroLogix, Cat # KR219)
- Wash Buffer Salts (makes 5L total)
- Substrate
- Stop Solution

### Materials Needed:

- Seed Extraction Buffer (if testing seed):
  - 35X Seed Extraction Buffer (add PVP + water to make 17.5L); Cat.# KR 186; AND
  - PVP (Sigma/PVP-40, CAS #9003-39-8 or equivalent)
- ~ OR ~
- 10X Seed Extraction Buffer (add water, makes 500 mL [sm] or 10L [lg]); Cat # KR 160
- 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 Seed Extraction Buffer
- seed grinding equipment  
(continued next page)

Catalog Number AP 062 V10

## 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 100 seeds). EnviroLogix' family of cucurbit ELISA kits (SqMV, CGMMV and MNSV) utilize identical extraction and testing protocols.

Because leaf tissue can have a higher expression of virus, an alternate SqMV protocol using a Conjugate Dilution step is presented (page 4) that better differentiates virus levels in leaf samples.

## Preparation of Solutions

- **Wash Buffer:** Add the contents of one 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 Seed Extraction Buffer:** If testing seed, it will be necessary to purchase either 35X or 10X Seed Extraction Buffer Concentrate.
  - **10X** (Cat. # KR160, 50 mL or 1000 mL) is a complete reagent; dilution in water is the only preparation step required.
  - **35X** (Cat. # KR186, 100 mL or 500 mL) requires the addition of Reagent Grade polyvinylpyrrolidone (PVP, CAS #9003-39-8) with average molecular weight of 40,000 (Sigma PVP40 or equivalent).

**Preparing 1X Seed Extraction Buffer:** Both concentrates are labeled with an expiration date at refrigerated storage. If ambient temperatures are continuously controlled within 18 to 25°C, the product will remain stable at room temperature up to the labeled date; any exposure to higher temperatures will void the predicted shelf life. The salts in the concentrates do precipitate when refrigerated. For this reason, it is highly recommended that an entire bottle of concentrate be used when preparing 1X buffer. If less than an entire bottle is used, all precipitated salts **MUST** be in solution before measuring out the concentrate; gentle warming while stirring may be necessary to achieve this.

- **10X preparation:** Add a 50 mL bottle of 10X Seed Extraction Buffer to 450 mL distilled / deionized water in a suitable container. Or add a 1000 mL bottle of 10X to 9L of distilled or deionized water in a suitable container. Mix thoroughly to dissolve any remaining precipitates. Refer to table for mixing alternate volumes.

- leaf extraction equipment
- centrifuge (optional, for leaf extracts)
- wash bottle, or microtiter plate or strip washer
- platform orbital plate shaker with orbital diameter of  $\geq 18$ mm, set to 150-200 rpm (do not use "microplate shakers" [ $< 5$  mm])
- microtiter plate reader or strip reader capable of reading 450 nanometers (nm)



Prepare wash buffer and extraction solutions



Add Extraction Buffer, controls, and sample extracts



Mix plate, incubate



All incubation steps must be performed on an orbital shaker with 18+ mm orbital diameter

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)

- **35X Preparation:** Add 350g of polyvinylpyrrolidone (PVP40) to 17L of distilled or deionized water in a suitable container. Mix thoroughly to dissolve the PVP into solution. Once the powder is completely dissolved, add one 500 mL bottle (lg.) of 35X Seed Extraction Buffer to the 17L solution. Or add 50g of PVP-40 to 3.5L of distilled or deionized water in a suitable container. Mix thoroughly to dissolve the PVP into solution. Once the powder is completely dissolved, add one 100 mL bottle (sm.) of 35X Seed Extraction Buffer to the 3.5L solution. Mix well. Refer to table for mixing alternate volumes.

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

Store 1X Seed Extraction Buffer refrigerated when not in use; warm to room temperature\* prior to assay.

**\*Please note: "room temperature" notation in all instructions is 18-25°C – do not expose kit components or solutions to temperatures above 25°C.**

## Sample Preparation

**Seeds:** The sample must be extracted with prepared 1X Seed 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 1X Seed Extraction Buffer
- 0.25 g of seed : 2.5 mL of 1X Seed Extraction Buffer

All seeds must be thoroughly ground/cracked in order for the internal tissue to come in contact with the buffer. Use equipment appropriate for the size of seed being tested (e.g. Polytron, coffee grinder, rubber mallet with mesh extraction bag, etc.). Soak ground seed tissue in 1X Seed Extraction Buffer for 1 hour minimum at 4°C. Solids will settle to the bottom; use the light-colored upper layer in the assay.

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

- 0.1 g of leaf : 1 mL of 1X Leaf Extraction Buffer

All leaf tissue must be thoroughly macerated in order for ideal sample extraction (e.g. EnviroLogix ACC 002 tube and pestle, mesh extraction bags, bead-beater apparatus). Note: extracts will be foamy.

Pull off particle-free extract to run in the test. Clarification of extracts by centrifugation is recommended (10 minutes at 1800-5000 x g), but not required.



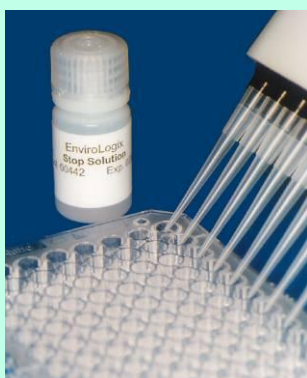
*Bottle Wash method*



*Add conjugate, mix, incubate, wash*



*Add substrate, mix, incubate*



*Add Stop Solution*



*Read plates in a Plate Reader  
at 450 nm  
within 30 minutes of the  
addition of Stop Solution.*

## 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 (18-25°C) - do not remove plates 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.
- Once all components have reached room temperature, remove the plate from the pouch. If less than the entire plate is to be used, refer to EnviroLogix' Product Application Guide - multiple runs on solid plate (available at [www.envirologix.com](http://www.envirologix.com) or call Tech Service).
- 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 Seed or Leaf Extraction Buffer 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.

1. Add **100 µL** of **1X Extraction Buffer**, **100 µL** of any **user-prepared negative control extract**, and **100 µL** 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, 4, 7 and 8.

2. Cover the wells with tape or Parafilm to prevent evaporation and incubate for **30 minutes** at **ambient temperature** on an **orbital shaker (with 18+ mm orbital diameter) at 150 to 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.

*Seed testing 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.*

3. 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 step, keep the plate inverted and tap firmly on a dry paper towel to remove as much Wash Buffer as possible.

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

4. Add **100 µL** of **Squash Mosaic Virus Enzyme Conjugate** to each well.
5. Cover the wells with new tape or Parafilm and **incubate** for **1 hour** at **ambient temperature** on an **orbital plate shaker as described above**. Note: Shaking during incubation steps is mandatory where called for. Failure to do so will result in up to 50% loss in assay sensitivity.
6. Wash the wells again as described in step 4. Alternatively, perform four washes (300 µL/well) with a microtiter plate or strip washer.



7. Add **100 µL** of **Substrate** to each well. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for 20-30 seconds. Cover the wells with new tape or Parafilm and incubate for **30 minutes** (for best results) at **ambient temperature**.
8. Add **100 µL** 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 between 600 and 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 Seed or Leaf 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.

## 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 4-8°C (39-46°F) when not in use.
- Do not expose QualiPlate components to temperatures greater than 37°C (99°F) or less than 2°C (36°F) for optimum performance.
- Allow all reagents to reach ambient temperature (18-25°C) 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 type or different lot number.

- 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.



**For Technical Support  
Contact Us At:**

**EnviroLogix**  
500 Riverside Industrial  
Parkway  
Portland, ME 04103-1486 USA

**Tel: (207) 797-0300**  
**Toll Free: 866-408-4597**  
**Fax: (207) 797-7533**

e-mail:  
[horticulture@envirologix.com](mailto:horticulture@envirologix.com)

website:  
[www.envirologix.com](http://www.envirologix.com)



## LIMITED WARRANTY

EnviroLogix Inc. (“EnviroLogix”) warrants the products sold hereunder (“the Products”) against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. If the Products do not conform to this Limited Warranty and the customer notifies EnviroLogix in writing of such defects during the warranty period, including an offer by the customer to return the Products to EnviroLogix for evaluation, EnviroLogix will repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

ENVIROLOGIX MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EnviroLogix products appearing in EnviroLogix published catalogues and product literature are EnviroLogix’ sole representations concerning the Products and warranty. No other statements or representations, written or oral, by EnviroLogix’ employees, agents or representatives, except written statements signed by a duly authorized officer of EnviroLogix Inc., are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

EnviroLogix does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by EnviroLogix, or against damages resulting from such non-EnviroLogix made products or components. EnviroLogix passes on to customer the warranty it received (if any) from the maker thereof of such non-EnviroLogix made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by EnviroLogix.

**THIS WARRANTY IS EXCLUSIVE.** The sole and exclusive obligation of EnviroLogix shall be to repair or replace the defective Products in the manner and for the period provided above. EnviroLogix shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall EnviroLogix be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

*Parafilm is a registered trademark of American Can Corporation  
EnviroLogix, the EnviroLogix logo, and QualiPlate are trademarks  
of EnviroLogix Inc.*

© EnviroLogix 2012