QualiPlate™ Kit for Lettuce Mosaic Virus

Catalog Number AP 059

Highlights:

- *Ready-coated plate*
- *One and a half hour (leaf/seedling) or two hour (seed) protocols*
- *Consistent quality*
- *Higher efficiency*
- *High sensitivity seed protocol option*

Contents of Kit:

- *Anti-Lettuce Mosaic Virus antibody-coated solid plate*
- *Lettuce Mosaic Virus Enzyme Conjugate*
- *10X Seed Extraction Buffer (makes 1 liter of 1X Seed Extraction Buffer)*
- *1X Leaf Extraction Buffer (50 mL; additional may be purchased from EnviroLogix, Cat # KR219)*
- *Packet Wash Buffer Salts*
- *Substrate*
- *Stop Solution*

Materials Needed:

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

Intended Use

The QualiPlate Kit for Lettuce Mosaic Virus screens for the presence of Lettuce Mosaic Potyvirus (LMV) in seed, leaf or seedling extracts obtained by different grinding methods. The antibody used in the kit has been shown to be reactive to at least six geographical isolates of the virus. In studies on seed lots determined to be LMV positive by other test methods, this kit was able to consistently detect the presence of the virus (using minimum sample sizes of 30,000 seeds and minimum sub-sample sizes of 500 seeds). This kit also has an alternative high sensitivity seed protocol (see section in "Precautions and Notes").

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.

- **1X Seed Extraction Buffer:** Bring 10X Seed Extraction Buffer to room temperature*, then stir or shake to dissolve precipitates completely before proceeding. To make 1X Seed Extraction Buffer, add both 50 mL bottles of 10X Seed Extraction Buffer to 900 mL of distilled or deionized water in a suitable container, and mix thoroughly to dissolve any remaining precipitates. Store 1X Seed Extraction Buffer refrigerated when not in use; warm to room temperature* prior to assay. Additional 10X Seed Extraction Buffer may be purchased from EnviroLogix (Cat#KR160).

  ***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 1:15 (gram of seeds to mL of buffer). For example:

- 0.5 g of seed : 7.5 mL of 1X Seed Extraction Buffer
- 0.05 g of seed : 0.75 mL of 1X Seed Extraction Buffer

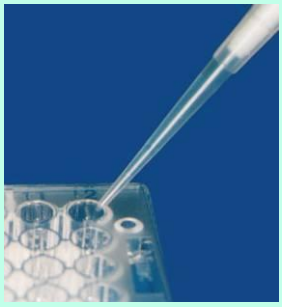
All seeds must be thoroughly ground/cracked in order for the internal tissue to come in contact with the buffer. Allow seed tissue to extract in buffer for 1 hour minimum at 4°C. Obtain a clear middle extract to run in the assay.

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.

Prepare wash buffer and extraction solutions



Add Extraction Buffer, controls, and sample extracts

Mix, incubate

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

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.

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

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

All seedling tissue must be thoroughly macerated in order for ideal sample expression, and should be tested immediately.

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 A1 or B1 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.
- 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 lettuce seed, leaf or seedling 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.

A. SEED PROTOCOL

A1. Add **100 µL** of **1X Seed Extraction Buffer, 100 µL** of any **user-prepared negative control seed 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 A1, A5, A8 and A9.

A2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for 4-5 seconds. Be careful not to spill the contents!

A3. 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.

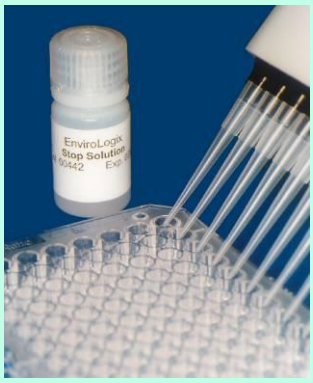
Protocol option: For testing convenience, at this point samples may be incubated overnight in the refrigerator (up to 16 hours at 5°C). Allow plates to come to room temperature with the rest of the kit reagents the next morning, before going on to step A4.

A4. After incubation, carefully remove the covering and empty the contents of the wells into a sink or other suitable container by

Bottle Wash method

Add conjugate, mix, incubate, wash

Add substrate, mix, incubate



Add Stop Solution

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

A5. Add **100 µL** of **Lettuce Mosaic Virus Enzyme Conjugate** to each well.

A6. 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 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**.**

A7. Wash the wells again as described in step A4. Alternatively, perform four washes (300 µL/well) with a microtiter plate or strip washer.

A8. 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**.

A9. 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.

B. LEAF & SEEDLING PROTOCOL

B1. Add **100 µL** of **1X Leaf Extraction Buffer, 100 µL** of any **user-prepared negative control leaf and/or seedling 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 B1, B5, B8 and B9.

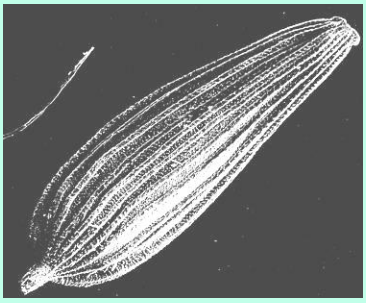
B2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for 4-5 seconds. Be careful not to spill the contents!

B3. 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**.**

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 B4.

B4. 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

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



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

B5. Add **100 µL** of **Lettuce Mosaic Virus Enzyme Conjugate** to each well.

B6. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate** for **30 minutes** 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**.**

B7. Wash the wells again as described in step B4. Alternatively, perform four washes (300 µL/well) with a microtiter plate or strip washer.

B8. 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**.

B9. 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.

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, leaf or seedling extract wells, to determine presence or absence of Lettuce Mosaic Virus in your sample extract. Samples with absorbances significantly greater than those of the Leaf or Seed Extraction Buffer and/or negative extract wells are presumed to be positive for Lettuce Mosaic Virus.

General Guidelines:

- Mean OD of Extraction Buffer wells should not exceed 0.10.
- Mean OD of LMV-free lettuce seed, leaf or seedling extracts should not exceed 0.15.

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

Note: This test kit, especially when following the high sensitivity protocol, is capable of detecting extremely low levels of LMV contamination.

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.
- 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.
- **High Sensitivity Seed Protocol.** This kit can be used with an alternative extraction buffer to increase its sensitivity up to tenfold. Prepare and use the High Sensitivity Extraction Buffer as directed below, and substitute it for the 1X Seed Extraction Buffer called for in the Seed Sample Preparation and Seed Assay Protocol sections.

  <u>High Sensitivity Seed Extraction Buffer</u>:

  In 200 mL of deionized water, dissolve:

	<u>Per Liter</u>
Sodium Phosphate (Na_2HPO_4) Dibasic anhydrous	5.4 g
Sodium Phosphate (NaH_2PO_4) Monobasic anhydrous	1.56 g
Thioglycolic Acid* ($C_2H_4O_2S$)	1.0 mL

  Bring volume up to a liter.
  Adjust pH to 7.4 using 8 M, NaOH.
  Prepare just prior to use.

  * SIGMA, T3758, Thioglycolic acid minimum 98%

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