QualiPlate™ Kit for Tomato Spotted Wilt Virus (TSWV)

Catalog Number AP 063

Contents of Kit:

- *Anti-Tomato Spotted Wilt Virus antibody-coated strip plate (12 strips of 8 wells each, in frame)*
- *Tomato Spotted Wilt Virus Enzyme Conjugate*
- *1X Leaf Extraction Buffer*
- *Packet Wash Buffer Salts*
- *Substrate*
- *Stop Solution*

Materials Needed:

- *pipettes capable of delivering 100 µL*
- *marking pen (indelible)*
- *tape or Parafilm®*
- *timer*
- *distilled or deionized water for preparing Wash Buffer*
- *microtiter plate reader or strip reader capable of reading at **450 nanometers** (nm)*
- *wash bottle, or microtiter plate or strip washer*
- *multi-channel pipette that will measure 100 µL*
- *racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)*
- *leaf extraction equipment*
- *centrifuge (optional)*
- *platform orbital plate shaker with orbital diameter of ≥18mm, set to 150-200 rpm (do not use "microplate shakers" [<5 mm])*

Prepare wash buffer

Intended Use

The QualiPlate Kit for TSWV screens for the presence of Tomato Spotted Wilt Virus (TSWV) in leaf extracts. The antibody used in the kit has been shown to be reactive to several geographical isolates of the virus. In studies on leaf tissue determined to be positive for TSWV by other test methods, this kit was able to consistently detect the presence of the virus.

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 formulae may be prepared from salts on site.

***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

Allow Leaf Extraction Buffer to come to room temperature before using.

The sample must be extracted with 10 volumes (mL/gram) of Leaf Extraction Buffer. For example:

- 0.1 g of leaf tissue : 1.0 mL of Extraction Buffer

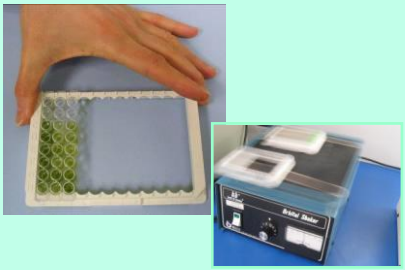
All leaf tissue must be thoroughly macerated in order for ideal sample extraction (e.g. EnviroLogix ACC 002 tube and pestle, mesh extraction bags, bead-beater apparatus). Note: extracts will be foamy.

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.

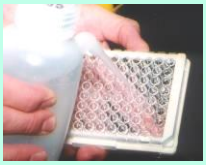
How to Run the Assay

- 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 in steps 1, 5, 8 and 9.
- Once all components have reached room temperature, remove the plate from the pouch. If fewer than all twelve strips are used, reseal the remaining strips and the desiccant in the foil pouch 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

Remove unneeded strips



Add Extraction Buffer and sample extracts; mix; incubate



Bottle Wash method

Add conjugate, mix, incubate, wash

Add substrate, mix, incubate

wells each of Leaf Extraction Buffer and a known-negative tomato 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 **Extraction Buffer**, **100 µL** of any **user-prepared negative control leaf 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, 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 4-5 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 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). Incubating overnight will result in lower relative results to same-day testing. 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 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 eight (8).

5. Add **100 µL** of **Tomato Spotted Wilt 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 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.

7. Wash the wells again as described in step 4. Alternatively, perform four washes (300 µL/well) with a microtiter plate or strip washer.

8. 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**.

9. 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.

Add Stop Solution

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

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

General Guidelines:

- Mean OD of Extraction Buffer wells should not exceed 0.10.
- Mean OD of TSWV-free tomato leaf extracts should not exceed 0.15.

If your 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°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.

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