QualiPlate™ Kit for Soybean Rust

Catalog Number AP 107

Contents of Kit:

- *Anti-Soybean Rust antibody-coated 12 x 8 strips in plate frame*
- *Soybean Rust Kit Positive Control (NOTE: does not contain actual soybean rust)*
- *Soybean Rust Enzyme Conjugate*
- *5x Soy Leaf Extraction Buffer*
- *Packet Wash Buffer Salts*
- *Substrate*
- *Stop Solution*

Early stages of rust

Later stages of rust

Prepare wash buffer and extraction solutions

Intended Use

The ELISA screens for the presence of soybean rust caused by *Phakopsora pachyrhizi*. The test can detect the presence of the pathogen at the very early stages of infection, from chlorotic lesions (before formation of a pustule) to immature pustules (not releasing spores). During this period it is often difficult and critical to differentiate the soybean rust symptoms from other diseases caused by bacterial, viral or fungal infections and/or insect damage. In addition, the test can also be used to detect advanced rust symptoms with uredinospores and teliospores, complementing visual inspections.

In controlled inoculation studies with levels as low as 100,000 spores/mL, this kit has been shown to detect the presence of soybean rust infection before the appearance of visual symptoms. Infection levels in the field may vary depending on environmental conditions.

Materials Needed

- Tissue Extraction Kit, EnviroLogix Cat. # ACC 002 *OR* Multi-Wall Mesh Pouch, EnviroLogix Cat. # ACC 021
- pipettes capable of delivering 100 µL
- marking pen (indelible)
- tape or Parafilm®
- timer
- distilled or deionized water for preparing Wash Buffer and for diluting 5x Soy Leaf Extraction Buffer
- glass bottles or flasks with 250 mL capacity for storage of 1x Soy Leaf Extraction Buffer and 1 liter capacity for Wash Buffer
- microtiter plate reader or strip reader
- wash bottle, or microtiter plate or strip washer
- multi-channel pipette that will measure 100 µL (optional)
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)
- orbital plate shaker (optional)

Preparation of Solutions

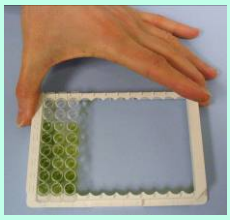
Wash Buffer:
Add the contents of the packet of **Wash Buffer Salts** (phosphate buffered saline, pH 7.4 - Tween 20) to 1 liter of distilled or deionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

1x Soy Leaf Extraction Buffer
To prepare, warm 5x Soy Leaf Buffer supplied with the kit to room temperature and mix well before diluting. Add one measure of 5x Buffer to


Punch leaf sample


Use pestle to mash leaf tissue


Remove unneeded strips


Add sample extract


Bottle Wash method

four measures distilled or deionized water in a suitable container to create 1x Soy Leaf Buffer. Mix thoroughly to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay. 1X Soy Leaf Buffer is stable for two weeks when stored refrigerated at 4-8°C and should be made on an as-needed basis. Please call EnviroLogix Tech Service if you have questions regarding calculating the appropriate amount of buffer to make for the number of samples that will be processed.

Sample Preparation

Sampling Recommendation:

Take a leaf sample that includes a suspect spot or area. The ACC 002 punch cap will result in a leaf sample of approximately 10mm diameter, weighing about 0.01 grams. If using an alternate method, care must be taken to ensure that the sample does not have an excess of non-affected tissue; this may reduce sensitivity.

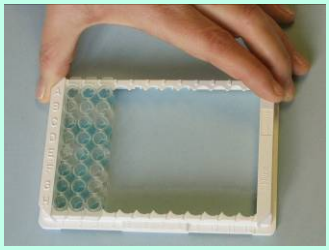
Sample Extraction:

1. Green leaf samples: Take a leaf punch sample by snapping the tube cap of the Disposable Tissue Extractor down on the leaf, encompassing the suspected rust spot. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Add 500 µl of Soy Leaf Extraction Buffer and continue grinding for 20-30 seconds or until the leaf tissue is well ground. *Use extreme caution to prevent sample-to-sample cross-contamination with plant tissue or exudate.*
2. Similar grinding devices (e.g. Multi-Wall Mesh Bags) can be used with a leaf to extraction buffer ratio (weight:volume) of 1:25 – 1:50 (eg. 3 mL to a 2.5 cm diameter leaf section). Using a hard object (e.g. coin) rub across the surface of the mesh bag against a hard surface until the leaf has transparent areas where the inside mesh has been forced through the leaf. After adding Extraction Buffer, massage the exterior of the mesh bag with fingers while holding the top of the bag closed to prevent the buffer from spilling.

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 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. If more than three strips are to be run at one time, the loading time will most likely exceed 15 minutes, and the use of a multi-channel pipette is strongly recommended in steps 1, 5, 8 and 9.
- If three or fewer strips are to be run, use a disposable-tip, air-displacement pipette and a clean pipette tip to add each Standard and 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 for these three reagents.
- 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.

Incubate



Test can be read visually, or…

…complete protocol and add Stop Solution

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

- 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 Blank (Extraction Buffer) and a known-negative soy leaf extract be run on each plate. Additional quality control samples may be added at the discretion of the user. The kit Positive Control is provided to show an example of a strong positive result. Sample extracts may be run in either single or duplicate wells.

1. Add **100 µL** of Extraction Buffer **Blank**, **100 µL** of the **Soybean Rust Kit Positive Control, 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.

   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 at ambient temperature for 1 hour.** If an orbital plate shaker is available shake plate at 200 rpm.

4. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with Wash Buffer, then shake to empty. Repeat this wash step three times.

5. Add **100 µL** of **Soybean Rust 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**. Use orbital shaker if available.

7. Wash the wells again as described in step 4. Alternatively, perform four washes (300 µL/well) with a microtiter plate or strip washer. Slap the plate on a paper towel to remove as much water as possible.

8. Add **100 µL** of **Substrate** to each well. Mix thoroughly as in step 2. Cover the wells with new tape or Parafilm and incubate for **20 minutes at ambient temperature**. Use orbital shaker if available.

NOTE: At this point, results may be scored visually. Test wells that are blue are positive for soybean rust. Very weakly positive (ie very light blue) results may require the use of a plate reader for confirmation. If the plate reader is to be used, continue with step 9.

Caution: Stop Solution is 1N Hydrochloric acid. Handle carefully.

9. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn any positive well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

Visual Inspection

After step 8 above, the well containing the Positive Control should show a distinct blue color. If not, that may indicate an invalid assay due to possible improper protocol, and the test should be repeated.

If after step 8 above, a test well is blue, this is interpreted as positive for *Phakopsora pachyrhizi*. Very weakly positive results may require the use of a plate reader for confirmation.

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 Blank wells, or preferably, to known-negative leaf extract wells, to determine presence or absence of soybean rust in your sample extract. Samples with absorbances significantly greater than those of the Blank and/or negative leaf extract wells are presumed to be positive for soybean rust.

Cross-Reactivity

The kit does not cross react with several other rust infections caused by *Uromyces, Puccinia* and *Melampsora* species. No cross reactivity was observed with other common fungal genera including *Aspergillus*, *Cercospora kikuchii* or *C. sojina, Fusarium*, *Penicillium*, *Peronospora mansurica*, *Pseudomonas savastanoi pv. Glycinea, Septoria, Rhizoctonia, Rhizopus, and Xanthomonas campestris pv. Glycinea.* No cross reactivity has been observed with similar-looking diseases such as frogeye leafspot, powdery mildew, downy mildew, brown spot, bacterial blight, bacterial pustule.

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).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) 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 expose Substrate to sunlight** during pipetting or while incubating in the test wells.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.

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