Mycelium Sample Protocol

Phytophthora ImmunoStrip®

Catalog number: 92601

YOU WILL NEED

- Sample extract buffer SEB1 (ACC 00996)
- Sample mesh bags (ACC 00930)
- Micropipettes
- Sterile micropipette tips
- Distilled water
- Conical micro tubes or microcentrifuge tubes

SAMPLE PREPARATION

Mycelium for the test can be harvested from agar, liquid and water culture.

Mycelium CMA-PARP medium

CMA-PARP (Snover-Clift, K., McKeller, M., and Chen. 2008) is the best agar medium to grow *Phytophthora* isolates to test with the ImmunoStrips. Other agar media can be used, but the culture needs a longer period of incubation.

The agar culture should be grown at least 10 days on CMA-PARP. Transfer 30 to 50 mg of mycelium scratched from the surface of the agar to a mesh bag, add 1 ml of SEB1, and grind the mycelium in the SEB1 buffer with the blunt tip of a marker. Small pieces of agar do not interfere with the test.

Mycelium agar from liquid culture

The culture grown in V-8 broth should be at least 5 weeks old. Transfer 30 to 50 mg of mycelium to a mesh bag, add 1 ml of SEB1, and grind the mycelium in the buffer with the blunt tip of a marker.

Supernatant from water culture

Dilute the water culture supernatant 1:100 in SEB1. For example, in a microcentrifuge tube mix 5 µl of water culture supernatant with 495 µl of SEB1. Insert the ImmunoStrip in the microcentrifuge tube. Optimum testing occurs from supernatant obtained closest to the mycelium.

Please note that the maximum volume of solution should range between 400-600 µl. Volumes outside of that range could produce false results.

TEST PROCEDURE

Remove the ImmunoStrip from the container. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove protective covering. Keeping the strips in a vertical position, insert the ends of the strips marked “sample” into the mycelium suspension contained in either the microcentrifuge tube or bag. Do not allow much more than 0.5 cm or ¼ inch of the ends of the strips to be submerged in the suspension. Be sure the strips remain in the suspension during the test

The control line will appear in 3 to 5 minutes. Maximum reaction occurs at 30 minutes at which time the ImmunoStrip should be removed from the suspension. The control line assures that the test is working properly. If the control line does not appear, the test is invalid. Refer to the product insert for interpreting results.

Note: Positive results can be observed after 5 minutes in samples with high antigen concentration.