Bt-Cry3Bb1 ImmunoStrip® Test

Strip tests for the detection of the Yieldgard® Rootworm resistance trait

Catalog no. STX 06100

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Size 0050	Item	Quantity
	ImmunoStrip®	50 strips
	Sample extract buffer (required)	Sold separately
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Size 0008	**Item**	**Quantity**
	ImmunoComb®, 12 strips per comb	8 combs
	Sample extract buffer (required)	Sold separately
	Instructions	1
Size 0012	**Item**	**Quantity**
	ImmunoComb®, 8 strips per comb	12 combs
	Sample extract buffer (required)	Sold separately
	Instructions	1

STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store container in the refrigerator (4°C) between uses. The sample buffer should also be refrigerated (4°C) when not in use.

YOU WILL NEED

- Scissors, pen, timer, and SEB4 sample extract buffer (ACC 01958)

Some of the items in the list below may be necessary depending on the type of samples and the method necessary to process the samples. Please refer to sample preparation section for guidance.

- Micropipettes and Micropipette tips
- Graduated cylinder
- Balance 1-500 gms
- 20% ethanol
- Micro tubes and tube rack
- Sample extraction bags (Agdia Catalog No. ACC00930) and bag stand
- Blender and accessories:
    - Blender (at least 450 watts) – optimal results were obtained using an Osterizer® blender at high speed (Sunbeam Corporation Model No. 6641, 1-800-597-5978)
    - Blender jars 125ml, Nalgene (“Mason” type, Fisher Scientific Catalog No. 11-815-10C)
    - Blender blade pack assembly (Oster® Sunbeam Product Catalog No. 4961)
    - Threaded bottom cap (Oster® Sunbeam Product Catalog No. 4902)

SAFETY

Sample extract buffer and strip tests are non-hazardous.

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INTENDED USE

This kit is intended for seed quality purposes to determine the presence of the Bt-Cry3Bb1 protein in seed and leaves of corn. The expression of Bt-Cry3Bb1 transgenic protein in plants results in the corn rootworm (CRW) resistance trait in corn. Bt-Cry3Bb1 acts as a biopesticide against the CRW complex (*Diabrotica* spp.).

This test system can be used to test individual corn seed and corn leaf or detect 1 transgenic Bt-Cry3Bb1 seed in 100 corn seeds (1%) and 1 transgenic Bt-Cry3Bb1 leaf in 100 corn leaves (1%). Validation testing at the 1% level produced a false positive and false negative error rate of 0%.

This test shows no cross-reaction with Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry1C, Bt-Cry1F, Bt-Cry2A, Bt-Cry3A, Bt-Cry9C or CP4 EPSPS (Roundup Ready®).

SAMPLE PREPARATION

Leaves, seedlings, or seeds must be ground and diluted in SEB4 sample extraction buffer. For best results, samples should be diluted in SEB4 buffer according to the ratios listed in the table below. After samples have been ground in buffer, let the extract sit for at least 1 minute before testing with the ImmunoStrip.

Leaf extraction

For leaf samples use Agdia's disposable sample extraction bags, a clean mortar and pestle, or any other grinding device to help extract samples.

Tissue	Ratio tissue to SEB4 buffer (weight/volume - g/ml)	Example
LEAF	1:10	0.3 g leaf: 3 ml buffer

Sample grinding in Agdia sample extraction bags

Individual leaves

A simple method for grinding a single leaf sample is by using Agdia's special sample extraction bags. Bags are available filled with buffer (Catalog No. ACC 00958) or empty (Catalog No. ACC 00930). Use only one sample per bag and be sure to label each bag. Add the appropriate volume of buffer to an empty bag or open one of the filled bags. Each filled bag contains 3 ml of sample buffer. A recommended 1:10 dilution, would require about a 0.3 g leaf sample. Place the sample between the mesh linings of the pouch. Rub the pouch with a pen to completely crush the sample and to mix the contents uniformly.

Cork borer and leaf disc

Multiple leaves

For composite leaf samples (up to 100 leaves), taking a representative leaf disc or leaf punch from each leaf is recommended. Several leaves can be stacked and punched. Place leaves on a clean surface and using a No. 2 cork borer (Fisher Scientific Catalog No. 07-854C) punch through the leaves. Dislodge the discs from the cork borer with a clean metal wire, weigh and transfer the discs into Agdia's disposable sample extraction bags and extract in buffer according to the recommended ratios. The weight of the discs varies with the growing conditions, age, and variety of the plant. Determine the average weight of discs and add the appropriate volume of SEB4 buffer.

Seed extraction

Note:

***Grinding:**
Seed grinding guidelines described in this instruction are optimized for a Osterizer® blender with a power rating of 450 watts. Blenders of lower power may require a longer grinding time. Other devices like coffee grinders or ball mills may also be used to grind the seeds. Visually check that the all seed has been ground to a fine powder.

Cleaning:
It is very important that the grinding equipment and workspace is cleaned well between each sample extraction. Wash blades, threaded caps and jars, with detergent making sure all ground material is washed away. Be especially careful to clean crevices of the blade. Any remaining powder can contaminate the next sample. After washing, wipe the grinding device and work area with 20% methanol.

Tissue	Ratio tissue to SEB4 buffer (weight/volume - g/ml)	Example
SEED	1:2.5	30 g seed: 75 ml buffer

Single seeds
Single seeds can be crushed with a seed crusher or hammer. Determine the average weight of the seed and add the appropriate volume of SEB4 buffer following a 1:2.5 (w/v) seed to buffer ratio. Let the extract sit for at least 1 minute before testing with the ImmunoStrip.

Multiple seeds
For composite seed samples (up to 100 seeds), it is recommended to use blender with a power rating of at least 450 watts in conjunction with "Mason" type jars.* The guidelines provided are optimized for Osterizer® blender with "Mason" type jars.

Put the seed sample in a dry "Mason" jar and assemble the blade attachment. A 125 ml jar is recommended for 100 seeds. Grind the seed at high speed for about 20 seconds or until all the seeds are ground to a powder. If using a 1000 ml jar, grind the seed for 60 seconds. Remove the jar from the blender and tap to collect all the powder. Shake the jar to mix and check for any unground seed.

Add the buffer at the specified ratio, close the lid and shake the bottle for 10-15 seconds. Let the extract sit for at least 1 minute before testing with the ImmunoStrip. Transfer 300 to 500 µl of the supernatant (top layer of liquid) to a clean micro tube for testing.

TEST PROCEDURE

Samples can be tested directly in the bag.

Remove the Bt-Cry3Bb1 strip 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 extract of the micro tube or sample extract bag. Do not allow much more than 0.5 cm or ¼ inch of the ends of the strips to be submerged in the extract. The end of the strip should remain in the extract during the test.

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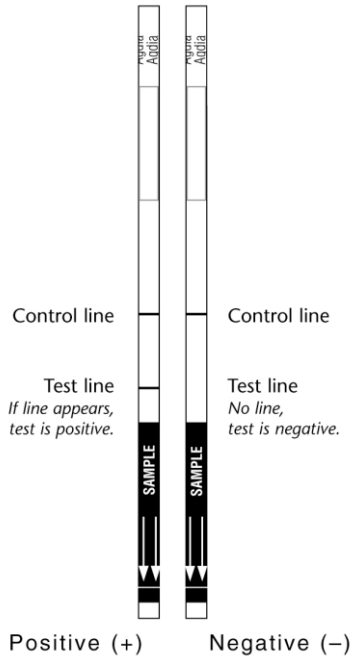
RESULTS



The control line can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 30 minutes at which time the ImmunoStrip should be removed from the buffer. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

If the sample is positive, the test line will also appear. If the sample is negative, the test line will not appear.

Do not remove the strip from the sample if control line is not visible. Leave the strip in the sample until the control line is visible and the sample flows into the wicking pad. Depending on the flow characteristics of the sample, the time to develop the signal may vary.

If you wish to keep the strips as permanent records blot the ImmunoStrips between paper towels. This prevents any liquid still in the sample pads from interfering with results.

LIMITATIONS

The following is a description of factors that could limit test performance or interfere with proper test results.

- Expiration: Test should be used within 1 year of purchase.
- Temperature: Optimal test results will occur when the test is run in an environment where the temperature is between 60º and 95º F (15º and 35º C).
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. If the ImmunoStrip package is left open too long, the strips may absorb moisture. This may affect test results.
- Sample Dilution: Strip performance is very dependent on the proper sample dilution. The strip will not properly absorb sample extracts containing large amounts of tissue.
- Submerging the strip: Test strips must not be submerged more than 0.5 cm or ¼ inch. If too much of the strip is submerged, certain components of the strip are released into the sample instead of being wicked upward by the strip. This most often results in a failed test in which no control line forms.