Bt-Cry34Ab1 ImmunoStrip® Test

Test for the detection of Bt-Cry34Ab1 transgenic protein
Catalog no. STX 04500

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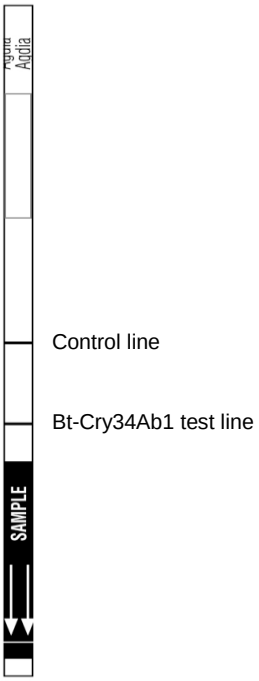
Size 0008	**Item**	**Quantity**
	ImmunoComb®, 12 strips per comb	8 combs
	Sample extract buffer (required)	Sold separately
	Instructions	1
Size 0012	**Item**	**Quantity**
	ImmunoComb®, 8 strips per comb	12 combs
	Sample extract buffer (required)	Sold separately
	Instructions	1



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. After removing the bottle from the refrigerator allow the bottle to warm up to room temperature before opening.

YOU WILL NEED

- Scissors, pen, timer, and SEB4 sample extract buffer
  SEB4 sample extraction buffer, available as:
  - SEB4 powder (ACC 01958/005.7) - Dissolve one bottle of powder into 1 liter of distilled water
  - SEB4 powder (ACC 01958/0050) - Dissolve one bottle of powder into 8.7 liters of distilled water
  - SEB4 powder (ACC 01958/0250) - Dissolve one bottle of powder into 43.8 liters of distilled water

- Sample extraction equipment
  - Transfer pipette or micropipettes and pipette tips
  - Graduated cylinder
  - Sample tubes, 1.5 ml and plastic pestle or a maceration device for grinding leaves
  - Weigh paper or wax paper and pliers for crushing single seeds
  - Blender and accessories for grinding multiple seeds:
    - 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 ImmunoStrip test is intended for seed quality purposes to determine the presence or absence of the insect resistance trait (Bt-Cry34Ab1) in transgenic corn. This test system can be used to test individual corn seed and corn leaf or detect 1 transgenic Bt-Cry34Ab1 seed in 799 corn seeds (0.125%)

The Bt-Cry34Ab1 ImmunoStrip has shown no cross-reaction with Bt-Cry1Ab, Bt-Cry1F, Bt-Cry3Bb1, Bt-Cry9C proteins, glyphosate tolerant events GA21 and NK603, or PAT/Liberty Link transgenic proteins.

TEST PROCEDURE

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 tables below. When handling the strips, always grasp the top of the ImmunoStrip® marked with the test name. Do not remove the protective covering.

Single Leaf Extraction

1. Make two leaf punches by folding a leaf in half and placing the fold between the body and cap of a 1.5 ml sample tube and snapping the cap into place.
2. Open the cap and remove the excess leaf tissue from around the opening. Push the leaf punches into the bottom of the tube with a plastic pestle.
3. Add about 0.4 ml of SEB4 buffer to the sample tube containing the leaf punches and macerate the leaf material with a plastic pestle until the solution turns light green.
4. Remove the Bt-Cry34Ab1 ImmunoStrip® from the container. Insert the end of the ImmunoStrip® marked “sample” into the extract of the sample tube. Allow the ImmunoStrip® to react for 15 minutes. The end of the ImmunoStrip® should remain in the extract during the test.
5. Remove ImmunoStrip® and interpret the results. See results section on page 4.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
LEAF	1:20	Two leaf disks (0.02 g): 0.4 ml buffer

Single Seed Extraction

1. Place a seed on a piece of weigh paper or waxed paper.
2. Fold the paper in over the seed and crush the seed into small pieces using pliers.
3. Put the crushed seed into a 1.5 ml sample tube and add 0.6 ml of SEB4 buffer.
4. Let the tube stand for 2 minutes while shaking intermittently or stirring with the stirrer.
5. Remove the Bt-Cry34Ab1 ImmunoStrip from the container. Insert the end of the ImmunoStrip® marked "sample" into the extract of the sample tube. Allow the ImmunoStrip® to react for 15 minutes. The end of the ImmunoStrip® should remain in the extract during the test.
6. Remove the ImmunoStrip® and interpret the results. See results section on page 4.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
SEED	1:2	1 seed (0.3 g): 0.6 ml buffer

Test Procedure continued

Composite seed Extraction

For composite seed samples (up to 800 seeds), it is recommended to use a 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.

1. Put the seed sample in a dry "Mason" jar and assemble the blade attachment. A 1000 ml jar is recommended for 800 seeds.
2. Grind the seed at high speed for about 60 seconds or until all the seeds are ground to a fine powder.
3. Remove the jar from the blender and tap to powder from the blade assembly. Add the buffer at the specified ratio, close the lid and shake the bottle vigorously for 45 seconds.
4. Let the extract sit for at least 1 minute before testing with the ImmunoStrip. Transfer 300 to 400 µl of the supernatant (top layer of liquid) to a clean micro tube for testing.
5. Remove the Bt-Cry34Ab1 ImmunoStrip from the container. Insert the end of the ImmunoStrip® marked "sample" into the extract of the sample tube. Allow the ImmunoStrip® to react for 15 minutes. The end of the ImmunoStrip® should remain in the extract during the test.
6. Remove the ImmunoStrip® and interpret the results. See results section on page 4.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
SEED	1:2	120 g seed powder : 240 ml buffer

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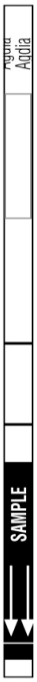
Note: *It is very important that the grinding equipment and workspace is cleaned well between each sample extraction. Wash blades, threaded caps, and jars with detergent making sure all ground material is washed away. Be especially careful to clean crevices of the blade. Any remaining powder can contaminate the next sample. Please note, the quality of the extraction as well as the extraction timing are minimums. More thorough extractions will lead to darker and more vivid test lines.*



Control line

Bt-Cry34Ab1 test line

Results

The control line can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 15 minutes at which time the ImmunoStrip should be removed from the sample extract. Use the image to the left as a guide to determine results. If necessary, align the ImmunoStrip with the image to determine the exact positions of the test lines and the control line.

The **control line** assures that the test is working properly. If the control line does not appear, the test is invalid and the test should be repeated.

If the sample is **positive** for the Bt-Cry34Ab1 trait, the **test line** will appear.

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

Note: If you wish to keep the ImmunoStrips as permanent records, cut off the sample pads (green ends marked "sample") and discard. This prevents any liquid still in the sample pads from interfering with results. Then blot the ImmunoStrips between paper towels.

Limitations

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

- Expiration: The ImmunoStrips® and SEB4 powder should be used within 1 year of purchase.
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. **The ImmunoStrip package must remain sealed with desiccant when not in use to prevent degradation of the ImmunoStrips® by moisture.**
- Sample Dilution: ImmunoStrip® performance is very dependent on the proper sample dilution. The ImmunoStrip® will not properly absorb sample extracts containing large amounts of tissue.
- Submerging the ImmunoStrip®: Test ImmunoStrips® must not be submerged more than 0.5 cm or ¼ inch. If too much of the ImmunoStrip® is submerged, certain components of the ImmunoStrip® are released into the sample instead of being wicked upward by the ImmunoStrip®. This most often results in a failed test in which no control line forms.
- Results: Some plant tissues may cause what appears to be a green test line. This may be due to the tissue type or to samples containing too much tissue. Samples producing such a result should be diluted further and retested. If the green line persists, contact Agdia directly for further assistance.

Technical Assistance

For technical assistance or questions regarding the use of this test system, please contact Agdia, Inc. Monday-Friday by phone at 1-800-622-4342. 1-574-264-2615 or by email at info@agdia.com.