

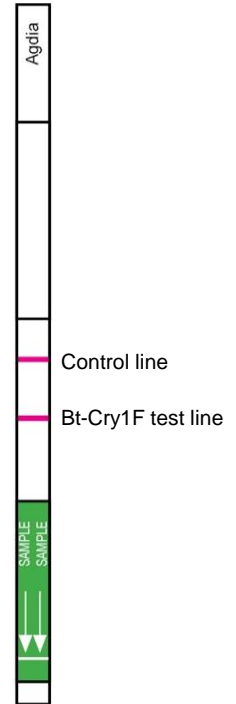
Bt-Cry1F ImmunoStrip® Test

Test for the detection of Bt-Cry1F transgenic protein

Catalog no. STX 10301

CONTENTS

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



STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store container refrigerated (2 – 8 °C) between uses. The 1X SEB4 should be refrigerated when not in use. After removing the bottle from the refrigerator, allow the bottle to warm to room temperature (18 – 30 °C) prior to opening.

YOU WILL NEED

- Scissors, pen, timer
- Single seed and leaf 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 leaf punches
 - Weigh paper or wax paper and hammer or rubber mallet for crushing single seeds
 - Or Seed crusher and plate (contact Agdia, Inc. for more information)
 - Tap water is required for single seed testing.
 - SEB4 sample extraction buffer is required for single leaf testing, 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
- Blender and accessories for grinding multiple seeds:
 - Tap water is required for composite seed testing.
 - 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 1000 mL, 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 to determine the presence or absence of the insect resistance trait Bt-Cry1F in corn. The Bt-Cry1F ImmunoStrip can be used for seed quality purposes, testing single seed or single leaf, as well as for grain testing. Currently this test is approved to detect 1 transgenic Bt-Cry1F seed in 200 corn seeds (0.5 %).

The Bt-Cry1F ImmunoStrip has shown no cross-reaction with Vip3A, GA21, Bt-Cry1Ab/1Ac, Bt-Cry2A, Bt-Cry3bb1, Bt-Cry1A.105, CSPB, Bt-Cry34, Amy797E, CP4, mCry3A, eCry3.1Ab, PAT/pat, AAD1, AAD12, DMO, 2mEPSPS or HPPD.

TEST PROCEDURE

Seed must be crushed and extracted with tap water. Leaves or seedlings must be ground in SEB4 sample extraction buffer. For best results, samples should be diluted in tap water or 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-Cry1F 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 5 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 SEB4

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 hammer or rubber mallet.
3. Put the crushed seed into a 1.5 mL sample tube and add 1.0 mL of tap water.
4. Vortex sample and let the tube stand for at least 1 minute.
5. Remove the Bt-Cry1F 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 5 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 tap water (weight/volume - g/mL)	Example
SEED	1 seed + 1.0 mL tap water	1 seed + 1.0 mL tap water

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Test Procedure Continued

Composite Seed Extraction

For composite seed samples (up to 200 seeds for corn), 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.
2. Grind the seed at high speed for 30 seconds. Dispense appropriate amount of tap water into jar, cap and shake vigorously for 30 seconds.
3. Let the extract sit for at least 1 minute before testing with the ImmunoStrip. Remove the cap and transfer 300 µL of the supernatant (top layer of liquid) to a clean micro tube for testing.
4. Remove the Bt-Cry1F 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 10 minutes. The end of the ImmunoStrip should remain in the extract during the test.
5. Remove the ImmunoStrip and interpret the results. See results section on page 4.

Tissue	Sample dilution with tap water (weight/volume - g/mL)	Example
CORN SEED	1:2	50 g seed powder: 100 mL tap water

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. Note: The qualities of the extractions as well as the extraction timing are the minimums. More thorough extractions will lead to darker and more vivid test lines.*

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RESULTS

The control line can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 5 minutes for single seed and leaf samples and 10 minutes for composite seed samples. 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-Cry1F 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 (colored 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 ImmunoStrips 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 (574-264-2014 or 800-622-4342) or by email (info@agdia.com).