Bt-Cry2A ImmunoStrip® Test

Strip test for the detection of Bt-Cry2A transgenic protein
Catalog no. STX 05801

CONTENTS

Size 0050	Item	Quantity
	ImmunoStrip®	50 strips
	Sample extraction buffer 4 (required)	Sold separately
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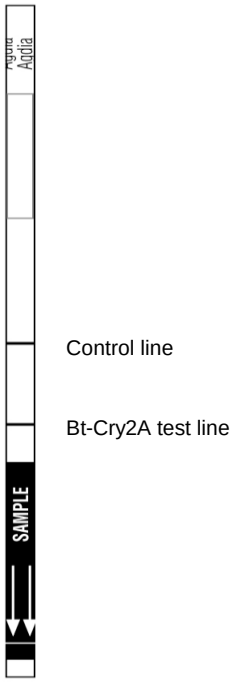
Size 0008	Item	Quantity
	ImmunoComb®, 12 strips per comb	8 combs
	Sample extraction buffer 4 (required)	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoComb®, 8 strips per comb	12 combs
	Sample extraction buffer 4 (required)	Sold separately
	Instructions	1



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
- Paper towels
- Timer
- SEB4 sample extraction buffer, available as:
  - Sample extract bag filled with SEB4 buffer (ACC 00958)
  - SEB4 powdered buffer (ACC 01958)
- Sample grinding device such as:
  - Agdia sample mesh bag (ACC 00930) and mallet
  - Seed press or seed crusher
  - Hammer or pliers
  - Analytical Balance
  - Variable volume pipette (1—500 µl range)
  - Pipette tips
  - Conical microtubes or conical microcentrifuge tubes (ACC 00340)

Safety

Sample extract buffer and strip tests are non-hazardous.

Technical Assistance

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

Intended Use

This ImmunoStrip® test is intended for seed quality purposes to determine the presence of the Bt-Cry2A protein in single seed and single leaf of corn.

The Bt-Cry2A ImmunoStrip® has shown no cross-reaction with other transgenic proteins in corn seed and leaf including Roundup Ready® Bt-Cry1Ab, Bt-Cry3Bb1, Bt-Cry9C, PAT/*pat* LibertyLink®, mBt-Cry3A, Bt-Cry34Ab1, Bt-Cry1F, GA21 and GT21.

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Sample Preparation

Sample ground in Agdia mesh extraction bag

Leaves or seeds must be ground and diluted in SEB4 extraction buffer. For best results, samples should be diluted in SEB4 buffer according to the ratios listed below. See the specific information below for each tissue type.

Leaf extraction

Individual leaves
A simple method for grinding a single leaf sample is by using Agdia's mesh sample bags. Use only one sample per bag and be sure to label each bag. Determine the weight of the leaf and place the leaf between the mesh linings of the extraction bag. Add the appropriate volume of SEB4 buffer to the bag. Rub the pouch with a pen to completely crush the sample and to mix the contents uniformly.

Another method would be making 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. 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. 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.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
LEAF	1:20	0.2 g leaf: 4.0 ml SEB4 buffer

Seed extraction

Single Seed
Single seeds can be crushed with a seed crusher, hammer or pliers. Determine the average weight of the seed and add the appropriate volume of SEB4 buffer.

For example, take a single seed and fold in weigh paper. Crush with pliers then transfer crushed seed to a conical microtube. Add the appropriate amount of SEB4 buffer, close the cap, and vigorously shake or vortex for 15 seconds. Allow the extract to settle for at least 1 minute before testing with the ImmunoStrip®.

If using Agdia's mesh sample bags, seed should be folded in the top portion of the bag and thoroughly crushed with a hammer. Crushed seed should be worked to the bottom of the bag. Add the appropriate amount of SEB4 buffer and mix the sample for at least 15 seconds

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
SEED	1:2.4	0.25 g: 0.6 ml SEB4 buffer

Test Procedure

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

Remove Bt-Cry2A strip from the container. Keeping strip in a vertical position, insert the end of the strip marked "sample" into the sample extract in the extraction bag. For extracts in microtubes, insert the end of the strip marked "sample" into the microtube making sure it is pushed gently into the tube as far as it will go. Do not allow much more than 0.5 cm or ¼ inch of the end of the strip to be submerged in the extract. The end of the strip should remain in contact with the extract for 5 minutes to allow for maximum reaction. Remove the ImmunoStrip® and interpret the results.

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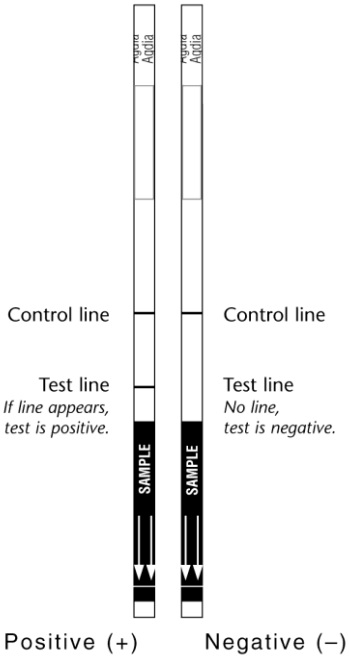
Results



The control line will appear in 3 to 5 minutes. Maximum reaction occurs in 5 minutes at which time the ImmunoStrip® should be removed from the sample extract. 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 cut off the sample pads and blot the ImmunoStrip® 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.