

Bt-Cry1F and Bt-Cry34Ab1 ImmunoStrip® Test

ImmunoStrip® test for the detection of Bt-Cry1F and Bt-Cry34Ab1 transgenic proteins
Catalog no. STX 10900

CONTENTS

Size 0050	Item	Quantity
	ImmunoStrip®	50 strips
	Sample extraction buffer 4 (required)	Sold separately
	Instructions	1
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

Control line
Bt-Cry1F line
Bt-Cry34Ab1 line



YOU WILL NEED

- Extraction buffer SEB4, powder (ACC 01958/005.7, ACC 01958/0050 and ACC 01958/0250)
- Sample extract pouch, SEB4 (ACC 00958)
- Sterile micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- Distilled or purified water
- Grinding equipment
 - Sample tube rack
 - Conical microtubes or conical microcentrifuge tubes (ACC 00340)
 - Pliers
 - Mesh sample bags (ACC 00930) and rubber mallet
 - Weigh paper

STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store container in the refrigerator (4°C) between uses. Liquid SEB4 should be refrigerated (4°C) when not in use.

SAFETY

Sample buffer and strip tests are non-hazardous.

INTENDED USE

This ImmunoStrip® test is intended for seed quality purposes to determine the presence or absence of the insect resistance traits (Bt-Cry1F and Bt-Cry34Ab1) HERCULEX® XTRA in transgenic corn.

The Bt-Cry1F and Bt-Cry34Ab1 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, GA21 and GT21.

Bt-Cry1F and Bt-Cry34Ab1 ImmunoStrip[®] Test

ImmunoStrip[®] test for the detection of Bt-Cry1F and Bt-Cry34Ab1 transgenic proteins
Catalog no. STX 10900

SAMPLE PREPARATION

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.

Sample grinding in Agdia sample extraction bags



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

It is important to use a conical microtube.



Seed extraction

Single seed

Single seeds can be crushed with pliers or rubber mallet. Determine the average weight of the seed and add the appropriate volume of SEB4 buffer.

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

Note:

It is very important to clean all the grinding equipment between the samples. Wash the equipment with detergent, rinse well, and completely dry with paper towel.

Bt-Cry1F and Bt-Cry34Ab1 ImmunoStrip® Test

ImmunoStrip® test for the detection of Bt-Cry1F and Bt-Cry34Ab1 transgenic proteins
Catalog no. STX 10900

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 ImmunoStrip® 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 10 minutes to allow for maximum reaction. Remove the ImmunoStrip® and interpret the results.

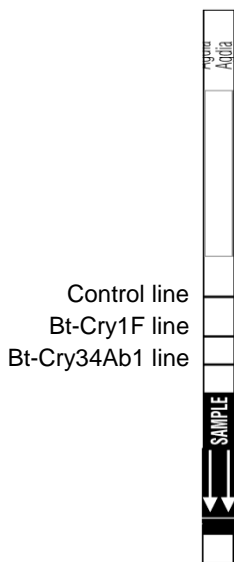
RESULTS

The control line will appear in 3 to 5 minutes. Maximum reaction occurs in 10 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 60° and 95° F (15° and 35° 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.

LibertyLink® is a registered trademark of Bayer.
HERCULEX® is a registered trademark of Dow AgroSciences LLC.
Roundup Ready® is a registered trademark of Monsanto.