

Quad Trait ImmunoStrip® Test

ImmunoStrip® test for the detection of Bt-Cry1F, Bt-Cry2A, Bt-Cry34Ab1 and Bt-Cry3Bb1 protein
Catalog number: STX 63200

CONTENTS

Size 0050	Item	Quantity
	ImmunoStrip®	50
	Extraction buffer, SEB7 liquid concentrate, 10X	Sold separately
	Instructions	1
Size 0008	Item	Quantity
	ImmunoComb®, 12 strips per comb	8 combs
	Extraction buffer, SEB7 liquid concentrate, 10X	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoComb®, 8 strips per comb	12 combs
	Extraction buffer, SEB7 liquid concentrate, 10X	Sold separately
	Instructions	1

YOU WILL NEED

- Extraction buffer SEB7, liquid concentrate 10X (ACC 00505)
- 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
 - Weigh paper

STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store container in the refrigerator (2 - 8 °C) between uses. After removing the bottle from the refrigerator allow the bottle to warm up to room temperature before opening.

SAFETY

Sample buffer and strip tests are non-hazardous.

INTENDED USE

This quad trait test is intended to determine the presence or absence of Bt-Cry1F, Bt-Cry2A, Bt-Cry34Ab or Bt-Cry3Bb1 transgenic proteins in corn seed or leaf.

This test has shown no cross reaction with Bt-Cry1Ab, CP4 EPSPS (Roundup Ready® corn), PAT/*pat* (Liberty Link®), mBt-Cry3A, GA21 and GT21 in corn seed. The traits contained on this strip have shown no cross reaction with each other.

LibertyLink® is a registered trademark of Bayer.

Roundup Ready® is a registered trademark of Monsanto.

TECHNICAL ASSISTANCE

For technical assistance or questions regarding the use of this test, please contact Agdia, by phone (800-622-4342 or 574-264-2014) or by email (info@agdia.com).

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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 one 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 storage instructions are not followed properly. The ImmunoStrip container must remain sealed with the desiccant when not in use to prevent degradation of the strips by moisture.
- 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.

SAMPLE PREPARATION



Sample ground in Agdia mesh extraction bag

Leaves or seeds must be ground and diluted in 1X SEB7 extraction buffer. For best results, samples should be diluted in 1X SEB7 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 1X SEB7 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 1X SEB7 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 1X SEB7 Buffer (weight/volume - g/mL)	Example
LEAF	1:20	0.2 g leaf: 4.0 mL 1X SEB7 buffer

Seed extraction

Single seed

Single seed samples should be crushed then transferred to a conical microtube or into the wells of a 24 or 48 microtiter plate. Add the appropriate amount of 1X SEB7 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 the microtiter plate, add the appropriate amount of 1X SEB7 buffer to each well. Place the plate on an orbital shaker at medium speed for three minutes. After

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incubation place ImmunoStrips into wells and read at 10 minutes.

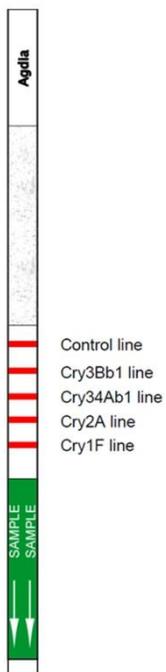
TISSUE TYPE	SAMPLE DILUTION in 1X SEB7 BUFFER (weight in grams : volume in mL)	EXAMPLE
Seed	1:3	0.250 g seed: 0.750 mL 1X SEB7 buffer

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 ImmunoStrip® from the container. Keeping strip in a vertical position, insert the end of the strip marked “sample” into the sample 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 can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 10 minutes at which time the ImmunoStrip should be removed from the sample. Use the image to the left as a guide to determine results. If necessary, align the strip with the image to determine exact positions of the test and control lines.

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 for a particular trait, the corresponding **test line** will appear. Multiple test lines may be present on the strip depending on samples being tested. If the sample is negative for a trait, the corresponding **test line** will not appear.

Note: If you wish to keep the ImmunoStrip as a permanent record, cut off the sample pad (colored end marked “sample”) and discard. This prevents any liquid still in the sample pad from interfering with results. Then blot the ImmunoStrip between paper towels.