

CP4 EPSPS, Bt-Cry1F and Bt-Cry1Ac ImmunoStrip® Test

ImmunoStrip® test for the detection of CP4 EPSPS, Bt-Cry1F and Bt-Cry1Ac transgenic proteins
Catalog number: STX 13200

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

Control line
Bt-Cry1Ac line
Bt-Cry1F line
CP4 EPSPS line



YOU WILL NEED

- Extraction buffer SEB4, powder (ACC 01958)
- Sample extract pouch, SEB4 (ACC 00958)
- Sterile micropipette tips
- Graduated cylinder
- Analytical balance
- 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. Allow container to warm to room temperature before opening. 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 of the Bt-Cry1F and Bt-Cry1Ac traits along with Roundup Ready® trait (CP4 EPSPS) in transgenic cotton seed and leaves.

This is a multi-analyte ImmunoStrip® that has three specific test lines. The Bt-Cry1F test line has shown no cross-reaction with other transgenic proteins in cotton seed and leaves including Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry2A, CP4 EPSPS (Roundup Ready®) or PAT/bar transgenic proteins.

The test line for Bt-Cry1Ac recognizes both Bt-Cry1Ac and Bt-Cry1Ab. It shows no cross reaction to Bt-Cry1F, Bt-Cry2A, CP4 EPSPS (Roundup Ready®) or PAT/bar transgenic proteins.

The CP4 EPSPS (Roundup Ready®) test line has shown no cross-reaction with Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry1F, Bt-Cry2A or PAT/bar transgenic proteins

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TECHNICAL ASSISTANCE

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 800-622-4342) or by email (info@agdia.com).

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. Allow sample to extract in buffer for 1 minute before testing with the ImmunoStrip®.

Sample grinding in Agdia sample extraction bags



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 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. Allow sample to extract in buffer for 1 minute before testing with the ImmunoStrip®.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
LEAF	1:20	0.15 g leaf: 3.0 ml SEB4 buffer

It is important to use a conical microtube.



Seed extraction

Single seed

Single seeds can be crushed in a 48 well plate with a seed crusher; then extracted with 0.5 ml SEB4. Shake on an orbital shaker at medium speed for 3 minutes. Allow sample to settle for 1 minute before testing.

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	1:10	0.10 g: 1.0 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 10 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 10 seconds. Allow seed to extract for 1 minute before testing with the ImmunoStrip®.

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.

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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.

When testing seeds in 48 well plates, insert the combed strips vertically down the back of the wells, gently push to the bottom.

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.

The control line will appear in 3 to 5 minutes. The test lines may appear in 3 to 5 minutes; however negative results should be confirmed in 10 minutes at which time maximum reaction occurs. ImmunoStrip® should then 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** for any of the proteins, a purple or red **test line** will appear. Use the diagram to the left to determine line positioning. Test line intensity can vary depending on the available antigen in the sample. Test lines appearing as green or gray should be considered inconclusive and should be retested.

If the sample is **negative** for a protein, no test line will appear for that protein.

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.

RESULTS

Control line
Bt-Cry1Ac line
Bt-Cry1F line
CP4 EPSPS line



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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 1 year of purchase.
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. If the ImmunoStrip® package must remain sealed with desiccant when not in use to prevent degradation of the strips by moisture.
- Sample: This strip is intended for testing black cotton seed only, treated cotton seed is not recommended.
- 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.
- Results: Some plant tissues may cause what appears to be a green or gray test line. This may be due to the tissue type or 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.

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