

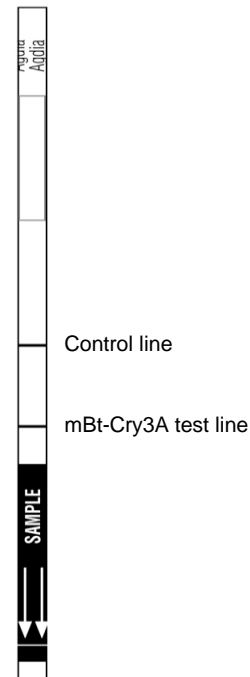
mBt-Cry3A ImmunoStrip® Test

Strip test for the detection of mBt-Cry3A transgenic protein

Catalog no. STX 06700

CONTENTS

Size 0050	Item	Quantity
	ImmunoStrip®	50 strips
	Sample extract buffer (required)	Sold separately
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Size 0008	Item	Quantity
	ImmunoComb®, 12 strips per comb	8 combs
	Sample extract buffer (required)	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoComb®, 8 strips per comb	12 combs
	Sample extract buffer (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. Allow the bottle to warm up to room temperature after removal from the refrigerator before opening.

YOU WILL NEED

- Timer
- **SEB4** sample extraction buffer, 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
- Sample extraction equipment
 - Sample tubes, 1.5 ml and plastic pestle or a maceration device for grinding leaves
 - Sample extract bag (ACC 00930)
 - Variable volume pipette (1 – 500 ul range)
 - Hammer or rubber mallet

SAFETY

Sample extract buffer and strip tests are non-hazardous.

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INTENDED USE

This ImmunoStrip® test is intended for seed quality purposes to determine the presence of the insect resistance trait (mBt-Cry3A) in transgenic corn. The test is appropriate for testing individual seeds and individual leaves. This protocol is not intended for composite seed testing.

The mBt-Cry3A ImmunoStrip® has shown no cross-reaction with Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry2A, Bt-Cry3Bb1, Bt-Cry9C, Bt-Cry1F, CP4 EPSPS (Roundup Ready®), or PAT transgenic proteins.

SAMPLE PREPARATION

Leaves, seedlings, or seeds must be ground and diluted in SEB4 sample extraction buffer. For best results, samples should be diluted in SEB4 buffer according to the ratios in each leaf and seed table. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove the protective covering.

Leaf extraction procedure:

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 discs 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 disks and macerate the leaf material with a plastic pestle until the solution turns light green.
4. Remove the mBt-Cry3A ImmunoStrip® from the container. Insert the end of the strip marked "sample" into the extract of the sample tube. Allow the strip to react for 10 minutes. The end of the strip should remain in the extract during the test.
5. Remove strip and interpret the results.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
LEAF	1:20	Two leaf disks (0.02 g): 0.4 ml buffer

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Single Seed extraction

Single seeds can be crushed with a seed crusher or hammer. Determine the average weight of the seed and add the appropriate volume of SEB4 buffer following a 1:2 (w/v) seed to buffer ratio.

Single Seed Procedure:

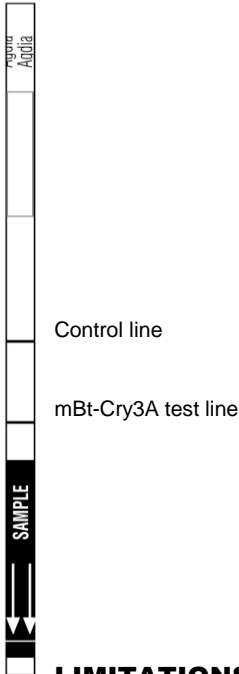
1. Place one seed in the center of a piece of paper. Fold the seed inside the paper. Place the wrapped seed on a hard surface and crush with a mallet or hammer. Simply cracking the seed into several large pieces is not sufficient. Crush the seed until it is thoroughly pulverized.
2. Transfer crushed seed into 1.5 ml centrifuge tube and add 0.6 ml SEB4 buffer.
3. Cap the tube and let it sit for at least 2 minutes. Then shake the contents of the tube for at least 15 seconds.
4. Remove the mBt-Cry3A ImmunoStrip® from the container. Insert the end of the strip marked "sample" into the extract of the sample tube. The end of the strip should remain in the extract during the test.
5. Read test after **30** minutes.

Tissue	Ratio tissue to SEB4 buffer (weight/volume - g/ml)	Example
SEED	1:2	1 seed (0.3 g): 0.6 ml buffer

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RESULTS



The control line can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 30 minutes at which time the ImmunoStrip® should be removed from the buffer. 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 mBt-Cry3A trait, the **test line** will appear.

If the sample is **negative**, the test line will not appear.

Note: If you wish to save ImmunoStrip® results, do so by scanning into an electronic image. Test lines can fade as the ImmunoStrip dries so strips should be scanned soon after testing.

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. **The ImmunoStrip® package must remain sealed with desiccant when not in use to prevent moisture degradation!** 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.

Roundup Ready® is a registered trademark of Monsanto Company

TECHNICAL ASSISTANCE

For technical assistance or questions regarding the use of this test system, please contact Agdia, Inc. Monday-Friday by phone at 1-800-622-4342 or by email at info@agdia.com.