

DMO, CP4 EPSPS, Bt-Cry2A, and Bt-Cry1Ac ImmunoStrip® Test

Catalog no. STX 42900



CONTENTS

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

INTENDED USE

The DMO, CP4 EPSPS, Bt-Cry2Ab, and Bt-Cry1Ac ImmunoStrips are to be used in transgenic cotton seed for the detection of the following traits: dicamba (DMO protein), Roundup Ready® Flex (CP4 EPSPS protein), and Bollgard II® (Bt-Cry2Ab and Bt-Cry1Ab/1Ac proteins).

This multi-analyte ImmunoStrip® has shown no cross-reactivity with AAD-12, Bt-Cry1F, Pat/bar, or Vip3A proteins.

Individual lines are specific for DMO, CP4 EPSPS, Bt-Cry2Ab and Bt-Cry1Ac.

Bollgard®, Bollgard II®, Roundup Ready® Flex, and XtendFlex® are registered trademarks of Monsanto Technology LLC.

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. Prepared 1X sample extraction buffers should also be refrigerated when not in use. After removing the bottle from the refrigerator allow the bottle to warm up to room temperature (18 - 30 °C) before opening.

YOU WILL NEED

- Scissors, pen, timer and transfer pipette or micropipettes and pipette tips
- Single seed extraction equipment
 - Sample mesh bags (ACC 00930)
 - Variable volume pipette (1 - 500 µL range) and pipette tips
 - Rubber mallet
 - Pliers
- **PBST** buffer is required for both **single seed** extractions:
 - Extraction buffer powder, PBST (ACC00115)
 - Extraction buffer 20X, PBST (ACC00501)

SAFETY

Sample extraction buffer and strip tests are non-hazardous.

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LIMITATIONS

The following is a description of factors that could limit test performance or interfere with proper test results.

- Expiration: The ImmunoStrips® 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 degradation of the strips by moisture.**
- Sample Dilution: ImmunoStrip® performance is very dependent on the proper tissue to buffer volume ratio. The ImmunoStrip® will not properly absorb sample extracts containing large amounts of tissue.
- Submerging the ImmunoStrip®: Test strips must not be submerged more than 0.5 cm or ¼ inch. If too much of the ImmunoStrip® 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 gray or green 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 gray or green line persists, contact Agdia directly for further assistance.

TECHNICAL ASSISTANCE

For technical assistance or questions regarding the use of this test system, please contact Agdia, Inc. by phone (1-800-622-4342 or 1-574-264-2014) or by email (info@agdia.com).

TEST PROCEDURE

Cotton seeds must be ground and diluted in sample extraction buffer according to the ratios listed below. Please note that seed samples should be used with PBST extraction buffer. PBST buffer is sold separately, see ACC00115 or ACC00501 for more information. When handling the strips, always grasp the top of the ImmunoStrip® marked with the test name. Do not remove the protective covering.

Single Seed Extraction

1. Place single cotton seeds into individual sample mesh bags.
2. Crush or pulverize the seeds using pliers. The seed should be powdered or broken into several pieces.

Tissue	Sample dilution with PBST buffer (weight/volume - g/mL)	Example
SEED	1:10	1 seed (0.1 g): 1.0 mL PBST

3. Determine the average weight of each seed and add the appropriate volume of **PBST** buffer following a 1:10 (tissue weight in g: buffer volume in mL) seed to buffer ratio.
4. Gently massage the bag for 10-15 seconds by hand.
5. Allow samples to extract in buffer for at least 1 minute before testing with ImmunoStrips®.
6. Insert the end of the strips marked "sample" into the sample extracts down the clear channel of each bag, it is important to keep the strip in a vertical position at all times. Allow the strip to react for 5 minutes. The end of the strip should remain in the extract during the test.
7. Another method for single seed extraction, utilizing the strips in comb format, would be placing single seeds in the wells of a 48 testwell microtiter plate. Using a seed crusher insert, thoroughly crush seeds with rubber mallet. Add 1.0 mL of PBST buffer to each well. Shake on an orbital shaker at medium speed for 5 minutes. Allow extract to settle for 1 minute before testing with the ImmunoStrip® comb.

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- When testing seeds in 48 well plates using combed ImmunoStrips, add the combed strips vertically down the back of the wells and gently push to the bottom of the wells.
- Remove the ImmunoStrips® and interpret results. After removal of sample pads, take a photograph of the results for your records.

RESULTS

Results should be interpreted after 5 minutes for single seed samples. The ImmunoStrip® should be removed from the sample extract. Use the image to the right 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 any of the proteins, a purple or red **test line** will appear. Test line intensity can vary depending on the available antigen in the sample. Test lines appearing as 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.

Note: If you wish to keep the ImmunoStrips® as permanent records, cut off the sample pads (colored ends marked “sample”) and discard. This prevents any liquid still in the sample pads from interfering with results. After removal of sample pads, take a photograph of the results for your records.

