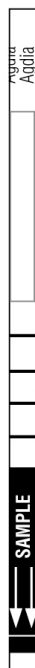


Bollgard[®] II (BG II) and Roundup Ready[®] (RR)

ImmunoStrip[®] test for CP4 EPSPS (RR), Bt-Cry2A, and Bt-Cry1Ac protein

Catalog number STX 51001



CONTENTS

Size 0050	Item	Quantity
	ImmunoStrip [®]	50 strips
	Sample extraction buffer (required)	Sold separately
	Instructions	1
Size 0008	Item	Quantity
	ImmunoComb [®] , 12 strips per comb	8 combs
	Sample extraction buffer (required)	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoComb [®] , 8 strips per comb	12 combs
	Sample extraction 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. Prepared 1X sample extraction buffers should also be refrigerated (4°C) when not in use. After removing the bottle from the refrigerator allow the bottle to warm up to room temperature before opening.

YOU WILL NEED

- Scissors, pen, timer and transfer pipette or micropipettes and pipette tips
- Single leaf extraction equipment
 - Agdia sample mesh bags or sample tubes (1.5 ml) and plastic pestle
 - **SEB4** sample extraction buffer is required for **single leaf** and is available as:
 - SEB4 buffer, powder (ACC 01958) - Dissolve 5.7 grams of powder into 1 liter of distilled water
 - SEB4 sample extraction bag (ACC 00958) - Mesh bag containing 3 ml SEB4 buffer
- Single seed extraction equipment
 - Sample mesh bags (ACC 00930)
 - Variable volume pipette (1 - 500 µl range) and pipette tips
 - Rubber mallet
 - Pliers
 - **SEB4** sample extraction buffer is required for **single seed** and is available as:
 - SEB4 buffer, powder (ACC 01958) - Dissolve 5.7 grams of powder into 1 liter of distilled water
 - SEB4 sample extraction bag (ACC 00958) - Mesh bag containing 3 ml SEB4 buffer

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[®] and sample extraction buffers 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 sample dilution. 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 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.

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 1-574-264-2615) or by email (info@agdia.com).

INTENDED USE

The BG II & RR ImmunoStrips are to be used for the detection of transgenic cotton seed and leaves containing the Roundup Ready[®] trait (CP4 EPSPS protein) and Bollgard[®] II trait (Bt-Cry2A and Bt-Cry1Ac proteins).

This is a multi-analyte ImmunoStrip[®] that has three specific test lines. The test line for Bt-Cry1Ac recognizes both Bt-Cry1Ac and Bt-Cry1Ab. It shows no cross reaction to Bt-Cry1F, Bt-Cry2A, BXN, CP4 EPSPS (Roundup Ready[®]) or PAT transgenic proteins.

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

The CP4 EPSPS (Roundup Ready[®]) test line has shown no cross-reaction with Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry1F, Bt-Cry2A, BXN or PAT proteins.

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TEST PROCEDURE

Cotton leaves or seeds must be ground and diluted in sample extraction buffer according to the ratios listed below. Please note that leaf and seed samples should be used with SEB4 extract buffer. When handling the strips, always grasp the top of the ImmunoStrip[®] marked with the test name. Do not remove the protective covering.

Single Leaf Extraction

1. For cotton leaf samples use Agdia's disposable sample extraction bags, a clean mortar and pestle, or any other grinding device to help extract samples.
2. Determine the weight of the leaf and place the leaf into the extraction bag.

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

3. Add the appropriate volume of **SEB4** buffer following a 1:20 (tissue weight in g: buffer volume in ml) leaf to buffer ratio.
4. Grind the sample by rubbing the outside of the extraction bag with a pen until the sample is completely ground.
5. After the samples have been allowed to extract in buffer for at least 1 minute, remove the ImmunoStrips[®] from the container.
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. Remove the ImmunoStrips[®] and interpret the results. See results section on page 4.

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 SEB4 Buffer (weight/volume - g/ml)	Example
SEED	1:10	1 seed (0.1 g): 1.0 ml buffer

3. Determine the average weight of each seed and add the appropriate volume of **SEB4** 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. After the samples have been allowed to extract in buffer for at least 1 minute, remove the ImmunoStrips[®] from the container.
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. Remove the ImmunoStrips[®] and interpret the results. See results section on page 4.
8. 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 0.5 ml of buffer to each well. Shake on an

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orbital shaker at medium speed for 3 minutes. Allow extract to settle for 1 minute before testing with the ImmunoStrip[®] comb.

- 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.

RESULTS

Results should be interpreted after 5 minutes for single seed and leaf samples. The ImmunoStrip[®] should be removed from the sample extract. 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 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.

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. Then blot the ImmunoStrips[®] between paper towels.

