

NPTII ImmunoStrip® Test

ImmunoStrip® test for the detection of neomycin phosphotransferase II
Catalog no. STX 73000



CONTENTS

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Size 0050	ImmunoStrip®	50 strips
	Sample extraction buffer (as specified below)	Sold separately
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Size 0008	Item	Quantity
	ImmunoStrip® Comb, 12 strips per comb	8 combs
	Sample extraction buffer (as specified below)	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoStrip® Comb, 8 strips per comb	12 combs
	Sample extraction buffer (as specified below)	Sold separately
	Instructions	1
	NPTII test line	

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 strips and buffer from the refrigerator allow the containers to warm to room temperature before opening to avoid condensation.

YOU WILL NEED

- Scissors, pen, timer and transfer pipette or micropipettes and pipette tips
- Single leaf extraction equipment
 - Agdia sample mesh bags (ACC 00930)
 - Sample bag holder, a small letter holder can be used
 - Microcentrifuge tubes, 1.5 ml (ACC 00340) and plastic pestle
 - **PEB1** sample extraction buffer is required for **single leaf** and is available as:
 - PEB1 buffer, 10X concentrate (ACC 00890) - 50 ml of concentrate makes ½ liter of buffer
 - PEB1 sample extraction bag (ACC 00140) - Mesh bag containing 3 ml PEB1 buffer
- Single seed extraction equipment
 - Sample mesh bags (ACC 00930)
 - Sample bag holder, a small letter holder can be used
 - Variable volume pipette (1 - 500 µl range) and pipette tips
 - Rubber mallet
 - Weigh paper
 - 48 well microtiter plate
 - Pliers
 - **SEB4** sample extraction buffer is required for **single seed** and is available as: SEB4 powder (ACC 01958) - Dissolve 5.7 grams of powder into 1 liter of distilled water.

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 Buffer: The NPTII ImmunoStrip® must be used with PEB1 sample extraction buffer when testing cotton leaves. It is best to use SEB4 when testing cotton seed samples. Do not use buffers used in other kits.
- Sample Dilution: ImmunoStrip® performance is very dependent on the proper sample dilution. The ImmunoStrip® will not properly absorb sample extracts containing excessive 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 an excessive amount of 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

Roundup Ready® cotton expresses the selectable marker protein neomycin phosphotransferase (NPTII), while Roundup Ready® Flex cotton does not. This ImmunoStrip® was developed and validated to determine the presence or absence of NPTII protein in single seeds of Roundup Ready® cotton and to confirm the absence of the NPTII protein in single seeds of Roundup Ready® Flex cotton.

This ImmunoStrip® is appropriate only when testing varieties from the cotton event, MON1445/1698 (*Roundup Ready® cotton, which expresses the NPTII protein*) and varieties from the cotton event, MON88913 (*Roundup Ready® Flex cotton, which does not express the NPTII protein*). Testing any stacked-trait events, such as Bollgard II with Roundup Ready® cotton is not recommended. Only Roundup Ready® and Roundup Ready® Flex events listed are appropriate for testing. Composite testing of seeds and leaves is also not recommended.

The NPTII ImmunoStrip® test will detect only the NPTII protein and has shown no cross-reaction with PAT/bar, PAT/pat, Bt-Cry1Ac, Bt-Cry2Ab or Bt-Cry1F.

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TEST PROCEDURE

Leaves, seedlings, or seeds must be ground and diluted in sample extraction buffer according to the ratios listed below. Please note that leaf samples should be used with PEB1 buffer and seed samples should be used with SEB4 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

Sample grinding in Agdia sample extraction bags



Microcentrifuge tubes (ACC 00340) are ideal for making leaf punches.



1. 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 PEB1 buffer to the bag. Rub the pouch with a pen to completely crush the sample and to mix the contents uniformly.
2. 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 microcentrifuge tube and snapping the cap into place. Open the cap and remove the excess leaf tissue from around the opening. Mature cotton leaf punches of this method typically weigh 0.02 g. Push the leaf punches into the bottom of the tube with a plastic pestle. Add about 0.4 ml of PEB1 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 PEB1 Buffer (weight/volume - g/ml)	Example
LEAF	1:20	0.15 g leaf: 3 ml buffer

3. After the samples have been allowed to extract in buffer for at least 2 minutes, remove the NPTII ImmunoStrips® from the container.
4. Insert the end of the strips marked "sample" into the sample extracts down the clear channel of each bag. Allow the strip to react for 30 minutes. The end of the strip should remain in the extract during the test.
5. Remove the ImmunoStrips® and interpret the results.

Single Seed Extraction

1. 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 15 seconds.
2. Single seeds may also be folded in weigh paper and crushed with pliers. Transfer crushed seed to a conical microcentrifuge tube. Add the appropriate amount of SEB4 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®.
3. Another method for single seed extraction, utilizing the strips in the 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 appropriate amount of buffer to each well. Shake on an orbital shaker at medium speed for 3 minutes. Allow extract to settle for 1 minute before testing with the ImmunoStrip® comb.
4. Insert the end of the strips marked "sample" into the sample extracts down the clear channel of each bag. Allow the strip to react for 30 minutes. The end of the strip should remain in the extract during the test.

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5. Remove the ImmunoStrips® and interpret the results.

Tissue	Sample dilution with SEB4 Buffer (weight/volume - g/ml)	Example
SEED	1:5	1 seed (0.1 g): 0.5 ml buffer

RESULTS

The control line can appear in as little as 3 to 5 minutes. Maximum reaction occurs in 30 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 the NPTII trait, 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 green or gray should be considered inconclusive and should be retested.

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

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

