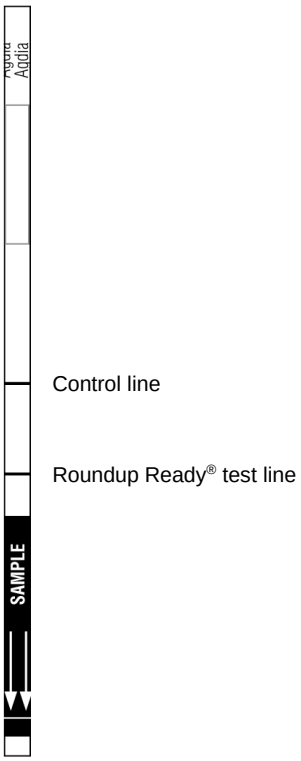
Roundup Ready® (CP4 EPSPS) ImmunoStrip® Test

Strip tests for the detection of CP4 EPSPS protein
Catalog no. STX 74000

Contents

Size 0050	Item	Quantity
	ImmunoStrip®	50 strips
	Sample extract buffer (required)	Sold separately
	Instructions	1
Size 0008	**Item**	**Quantity**
	ImmunoStrip® Comb, 12 strips per comb	8 Combs
	Sample extract buffer (required)	Sold separately
	Instructions	1
Size 0012	**Item**	**Quantity**
	ImmunoStrip® Comb, 8 strips per comb	12 Combs
	Sample extract buffer (required)	Sold separately
	Instructions	1



Materials required but not provided

Micropipettes
Sterile micropipette tips
Graduated cylinder
Balance 1-500 grams
SEB4 sample extraction buffer 4 (ACC 01958), optional
Scissors and a pen
Distilled water
Grinding equipment:
- Blender (Osterizer®, Sunbeam Corporation Model No. 6641, 1-800-597-5978)
- Blender jars 1000 mL, Nalgene (“Mason” type, Fisher Scientific Catalog No. 2115-1000)
- Blender blade packs assembly (Factory Services Inc. Catalog No. OC-DUX, 1-800-237-8699)
- Threaded bottom cap (Factory Services Inc. Catalog No. OJN)
- Plastic extraction bottles 1000 mL
- Sample tube rack
- Conical micro tubes or conical microcentrifuge tubes
- Sample extraction bags (ACC 00930)

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. The 1X SEB4 should be refrigerated when not in use. ImmunoStrips and extraction buffer should be warmed to room temperature (18 – 30 °C) prior to use.

Safety

Sample buffer and strip tests are non-hazardous.

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Intended Use

This kit is intended for grain testing and seed quality purposes to determine the presence of the CP4 EPSPS protein in seed and leaves of corn, cotton, and soybean. The expression of CP4 EPSPS transgenic protein in plants results in Roundup® herbicide resistance. Roundup® is a broad spectrum herbicide used to control weeds.

Currently this test is approved for use in cotton, corn, and soybean. Using this test system, you can reliably detect 1 transgenic CP4 EPSPS seed in 1000 seeds (0.1 %).

Sample Preparation

Leaves, seedlings, or seeds must be ground and diluted. Those testing for seed quality purposes should use SEB4 sample extraction buffer to dilute both leaves and seeds. When testing grain, water can be used to dilute samples. After samples have been ground and diluted, let the extract sit for at least 30 seconds before testing with the ImmunoStrip®. See the specific information below for each tissue type.

Leaf extraction

For leaf samples use Agdia's disposable sample extraction bags, a clean mortar and pestle, or any other grinding device to help extract samples.

Individual leaves

Sample grinding in Agdia sample extraction Bags

A simple method for grinding a single leaf sample is by using Agdia's special sample extraction bags. Buffer filled mesh bags (ACC 00958) or sample mesh bags, unfilled (ACC 00930) can be purchased from Agdia. Use only one sample per bag and be sure to label each bag.

Add the appropriate volume of buffer to the sample mesh bag. Prefilled bags contain 3 mL of sample buffer. Place the sample between the mesh linings of the pouch. Rub the pouch with a pen to completely crush the sample and to mix the contents uniformly.

Crop	Leaf to SEB4 buffer ratio (weight/volume)	Approximate sample weight	Volume of SEB4 Buffer
Corn	1:10	0.2 grams	2 mL
Cotton	1:20	0.2 grams	4 mL
Soybean	1:20	0.1 grams	2 mL

Seed extraction

Single seeds

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. Let the extract sit for at least 30 seconds 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. Wiping the grinding device and work area with 20 % methanol is also recommended between samples

Composite seed/grain sample

For seed / grain samples to be tested at 0.1 % sensitivity level, it is recommended to use Osterizer® blender with "Mason" type jars to accommodate 1000 seeds. However, depending on the sample size other devices like coffee grinders, ball mill, other blenders, or seed crusher may be used to grind the samples. The guidelines provided are optimized for Osterizer® blender with "Mason" type jars.

Put the seed sample in a dry "Mason" jar and assemble the blade attachment.
Grind the seed at high speed for about 45 to 60 seconds or until all the seeds are ground to a powder. Remove the jar from the blender and tap to collect all the powder. Shake the jar to mix and check for any unground seed.

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Transfer the ground powder to a container and weigh the specified amount (sub sample) from the following table to a 500 mL disposable bottle. Dilute the ground powder at the specified ratio, close the lid and shake the bottle for 10-15 seconds. Let the extract sit for at least 30 seconds before testing with the ImmunoStrip®. Use only the supernatant (top layer of liquid) for testing

Crop	Seed to buffer ratio (weight/volume)	Sub sample weight	Volume of SEB4 Buffer for seed quality testing	Volume of water for grain testing
Corn	1:2	50 grams	100 mL	100 mL
Cotton	1:10	20 grams	200 mL	200 mL
Soybean	1:5	20 grams	100 mL	100 mL

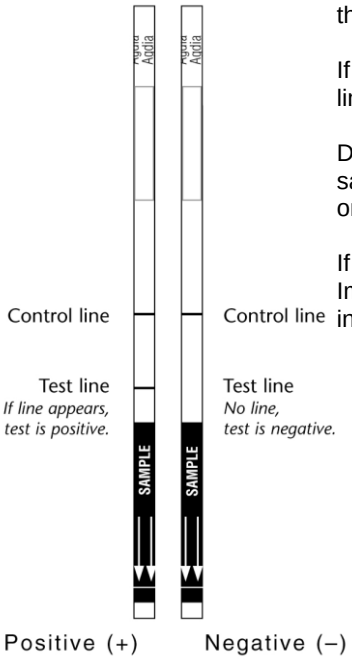
Test Procedure

Transfer 500 µL of extracted sample to a conical microtube. If Agdia sample extract bags were used for extraction, samples can be tested directly in the bag.

It is important to use a conical microtube.

Remove Roundup Ready® (CP4 EPSPS) strip from the container. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove protective covering. Keeping the strips in a vertical position, insert the ends of the strips marked “sample” into the extract of the microtube or bag. Do not allow much more than 0.5 cm or ¼ inch of the ends of the strips to be submerged in the extract. Be sure the strips remain in the extract during the test.

Results



The control line will appear in 3 to 5 minutes. Maximum reaction occurs in 20 minutes at which time the ImmunoStrip® should be removed from the buffer. 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, the test line will also appear. If the sample is negative, the test line will not appear.

Do not remove the strip from the sample if control line is not visible. Leave the strip in the sample until the control line is visible and the sample flows into the wicking pad. Depending on the flow characteristics of the sample, the time to develop the signal may vary.

If you wish to keep the strips as permanent records cut off the sample pads and blot the ImmunoStrips between paper towel. This prevents any liquid still in the sample pads from interfering with results.

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.
- Temperature: Optimal test results will occur when the test is run in an environment where the temperature is between 60º and 95º F (15º and 35º C).
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. The ImmunoStrips package must remain sealed with desiccant when not in use to prevent degradation of the ImmunoStrips by moisture.
- 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.