DMO ImmunoStrip® Test

ImmunoStrip® test for the detection of Dicamba transgenic proteins
Catalog no. STX 29800

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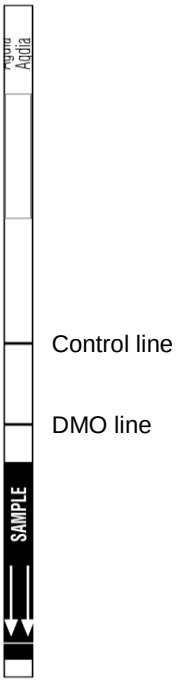
Size 0008	Item	Quantity
	ImmunoComb®, 12 strips per comb	8 combs
	Sample extraction buffer	Sold separately
	Instructions	1
Size 0012	Item	Quantity
	ImmunoComb®, 8 strips per comb	12 combs
	Sample extraction buffer	Sold separately
	Instructions	1



Control line

DMO line

YOU WILL NEED

- SEB4 sample extraction buffer powder (ACC 01958) for leaf testing
- Water for single seed and composite seed testing
- Micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- Grinding equipment
    - Sample tube rack
    - Conical microtubes or conical microcentrifuge tubes (ACC 00340)
    - Pliers
    - Mesh sample bags (ACC 00930) and rubber mallet
    - Weigh paper
    - Blender (at least 450 watts)-optimal results were obtained using an Osterizer® blender at high speed (Sunbeam Corporation Model Number 6641)
    - Blender jars 125 mL, Nalgene (“Mason” type, Fisher Scientific Catalog Number 11-815-10C)

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. 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.

INTENDED USE

This ImmunoStrip® test is intended for seed quality purposes to determine the presence or absence of DMO protein in transgenic soybean.

This test system can be used to test individual soybean and soybean leaf or detect 1 transgenic DMO seed in 400 soybean seeds (0.25 %).

The DMO ImmunoStrip® has shown no cross-reaction with other transgenic proteins in soybean seed and leaf including AAD-12, 2mEPSPS, PAT/pat, CP4 EPSPS, HPPD, Bt-Cry1Ac, and Bt-Cry1F.

SAMPLE PREPARATION

Leaves must be extracted in SEB4 buffer. Single seeds should be extracted in water, and composite seed should be ground and diluted in water. For best results, samples should be diluted according to the ratios listed below. See the specific information below for each tissue type.

Leaf extraction

Use the table below to determine the amount of buffer needed.

Tissue	Sample dilution in SEB4 buffer (weight in grams: volume in mL)	Example
LEAF	1:20	0.2 g leaf: 4.0 mL SEB4 buffer

Sample grinding in Agdia sample extraction bags

Individual leaves

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 SEB4 buffer to the bag. Rub the pouch with a pen to completely crush the sample and to mix the contents uniformly.

It is important to use a conical microtube.

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 sample tube and snapping the cap into place. Open the cap and remove the excess leaf tissue from around the opening. Push the leaf punches into the bottom of the tube with a plastic pestle. Add about 0.4 mL of SEB4 buffer to the sample tube containing the leaf punches and macerate the leaf material with a plastic pestle until the solution turns light green.

Seed extraction

Use the table below to determine the amount of buffer needed.

Tissue	Sample dilution in water (weight in grams: volume in mL)	Example
SEED	1:5	0.25 g seed: 1.25 mL water

Single seed

Single seed samples should be thoroughly crushed then transferred to a conical microtube or into the wells of a 24 or 48 microtiter plate. Add the appropriate amount of water, close the cap on the conical microtube, and vigorously shake or vortex for 15 seconds. Allow the extract to settle for at least 1 minute before testing with the ImmunoStrip®.

If using the microtiter plate, add the appropriate amount of water to each well. Place the plate on an orbital shaker at medium speed for three minutes.

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.

Composite Seed Extraction

Use the table below to determine the amount of buffer needed.

Tissue	Sample dilution in water (weight in grams: volume in mL)	Example
SEED	1:5	25 g seed: 125 mL of water

Composite Seed

For composite seed samples (up to 400 seeds), it is recommended to use a blender with a power rating of at least 450 watts in conjunction with “Mason” type jars. The guidelines provided are optimized for Osterizer® blender with “Mason” type jars.

1. Put the seed sample in a dry “Mason” jar and assemble the blade attachment.

2. Grind the seed at high speed for 30 seconds or until all the seeds are ground to a fine powder. Dispense appropriate amount of water into jar, cap and shake vigorously for at least 30 seconds.

3. Let the extract sit for at least five minutes, remove the cap and transfer 250 µL of the supernatant (top layer of liquid) to a clean micro tube, allow the extract in the micro tube to settle for one minute before insert the ImmunoStrip® for testing.

Note:

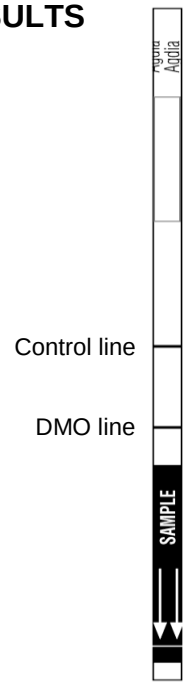
It is very important that the grinding equipment and workspace is cleaned well between each sample extraction. Wash blades, threaded caps, and jars with detergent making sure all ground material is washed away. Be especially careful to clean crevices of the blade. Any remaining powder can contaminate the next sample. Note: The qualities of the extractions as well as the extraction timing are the minimums. More thorough extractions will lead to darker and more vivid test lines.

TEST PROCEDURE

When handling the strips, always grasp the top of the ImmunoStrip® marked with the test name. Do not remove the protective covering.

Remove ImmunoStrip® from the container. Keeping strip in a vertical position, insert the end of the strip marked “sample” into the sample extract in the extraction bag. For extracts in microtubes, insert the end of the strip marked “sample” into the microtube making sure it is pushed gently into the tube as far as it will go. Do not allow much more than 1.0 cm or 3/8 inch of the end of the strip to be submerged in the extract. For maximum reactions, the end of the strip should remain in contact with the extract for 5 minutes for leaf or single seed and 10 minutes for composite seed. The control line will appear in 3 to 5 minutes. Remove the ImmunoStrip® from the sample extract and interpret the results.

RESULTS



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.

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.

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.

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 60 – 95 ºF (15 – 35 ºC).
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. If the ImmunoStrip® package is left open too long, the strips may absorb moisture. 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 1.0 cm or ⅜ 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.

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).