

USER GUIDE: AAD-12 ImmunoStrip[®] Test
Catalog number: 32200



KIT INFORMATION

Intended Use

This ImmunoStrip[®] test is intended for seed and leaf quality purposes to determine the presence or absence of the Enlist[™] trait (AAD-12) that is resistant to 2,4-D herbicide in transgenic soybean and cotton seed and leaf.

The test limit of detection is one (1) AAD-12 cotton seed in 100 non-transgenic cotton seeds (1.0 % LOD) and one (1) AAD-12 soybean in 200 non-transgenic soybeans (0.5 % LOD).

This ImmunoStrip does not cross-react with other transgenic proteins in cotton and soybean including CP4 EPSPS, PAT/*pat*, Pat/*bar*, Bt-Cry1Ac, Bt-Cry2A, Bt-Cry1F, 2mEPSPS, DMO, HPPD, GAT and Vip3A.

ImmunoStrip tests require no expertise to run. Results are obtained in as little as a few minutes making them perfect for use in the field. The ImmunoStrip **must** be used with tap water for composite seed and single seed testing and **1X SEB4** sample extraction buffer for single leaf. Do not use any other extraction buffer.

Storage of Kit

ImmunoStrips should be stored at room temperature (18 - 30 °C) between uses and tightly sealed in the desiccated container at all times.

SAFETY

ImmunoStrips are non-hazardous. Please refer to SDS for hazards associated with SEB4 buffer: <http://docs.agdia.com/DataSheets.aspx>

ImmunoStrips Include

- ImmunoStrips
- User guide

What's required to perform the assay?

- SEB4 buffer (ACC 01958)
- Micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- Distilled or purified water
- Grinding equipment
 - Sample tube rack
 - 1.5 mL conical microtubes or conical microcentrifuge tubes (ACC 00340)
 - Pliers
 - Mesh sample bags (ACC 00930) and rubber mallet (optional)
 - Weigh paper
 - Golf Tee or disposable pestle

Validated Sample Dilution Ratios and Diluents

Host	Sample Type	Dilution Ratio	Diluent	Example
Cotton / Soybean	Composite Seed	1: 6 (weight: vol – g: mL)	Tap water	10 g: 60 mL Tap water
Cotton / Soybean	Single Seed	1 seed : 1 mL	Tap water	1 seed: 1 mL Tap water
Cotton / Soybean	Single Leaf	1: 20 (weight: vol – g: mL)	1X SEB4	0.2 g leaf : 4mL 1X SEB4

PREPARING THE SAMPLE

Composite Seed

A variety of composite seed extraction methods can be used providing the seed is thoroughly ground in containers free of residual contaminants and extracted at 1:6 using Tap water. For this test, composite seed samples of up to 200 seeds for soybean and 100 seeds for cotton can be extracted per sample. Agdia recommends using an Osterizer[®] blender with 250 mL size “Mason” type jar or similar equipment following the procedure below.

1. Place the weighed 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 buffer 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 400 µL of the supernatant (top layer of liquid) to a clean 1.5 mL microtube (or similar). Allow the extract in the microtube to settle for 30 seconds before inserting the ImmunoStrip for testing.

Single Seed

A variety of single seed extraction methods may be used, providing that seeds are adequately crushed, extracted at 1 seed : 1 mL in tap water and then allowed to settle for at least 1 minute before testing with the ImmunoStrip®. Regardless of the method chosen, please be cognizant of potential cross contamination by seed particles.

Example methods:

- Single seed extraction 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 mL of tap water 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.
- Seed may be crushed 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, hydrated with 1 mL of tap water and massaged with your fingers for at least 15 seconds prior to settling and testing. Agdia recommends removing the top half of the mesh bag prior to testing.

Single Leaf

A variety of leaf tissue extraction processes may be used, providing the leaves are thoroughly macerated, extracted at 1:20 in 1X SEB4 and allowed to settle for at least 1 minute before testing with the ImmunoStrip.

Example methods:

- Agdia's mesh sample bags may be used with the sample size of your choice, normally between 0.1–0.3 g. The appropriate amount of 1X SEB4 is added and the leaf tissue is macerated by rubbing the pouch with a homogenizer or the end of a rounded blunt object. Agdia recommends removing the top half of the mesh bag prior to testing.
- Leaf tissue may also be extracted by cutting two leaf punches (~0.02 g) using the closing action of a 1.5 mL conical microtube and cap. Subsequently, 0.4 mL of 1X SEB4 is added to the sample and thoroughly macerated (10-15 sec.) with a golf tee or disposable pestle.

PERFORMING THE ASSAY

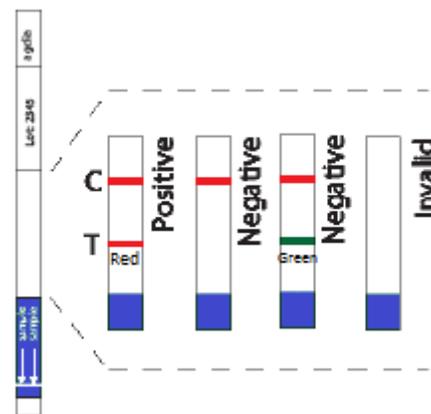
Insert the ImmunoStrip vertically into the microtube, or, if applicable, into the channel portion (no mesh) of the mesh bag. ***Be sure to insert the "sample" end of the strip into the extract no more than ¼" or up to the white line on the ImmunoStrip.**

Single Seed and Single Leaf: Remove the ImmunoStrip after 5 minutes of incubation with sample extract and interpret results immediately. Positive results may be visible in as little as 2 minutes.

Composite Seed: Remove the ImmunoStrip after 10 minutes of incubation with sample extract and interpret results immediately.

Any red or pink signal in the test line area must be interpreted as **positive**. Maximum reaction occurs in 5 minutes for single seed/leaf and 10 minutes for composite samples at which time the ImmunoStrip should be removed from the sample and interpreted. If the sample is **negative**, the test line will not appear. The control line assures that the test is working properly.

Note: If you wish to keep the ImmunoStrip result as a permanent record, please take a photo at the time of test to keep as a reference.



TROUBLESHOOTING

Control line did not develop.	This situation is generally caused by over-submergence of the test strip in the sample extract. If no control line is present, results should be considered invalid, and the test should be repeated.
Test runs very slow or not at all.	This can be caused by using too much tissue for extraction. Repeat the test using less tissue. If the above is not the case, make sure the test components were warmed to temperature before use and are within their expiration date.
Test has a green test line.	Green lines should not be considered as a positive result.
Test and/or control line is weak.	Make sure the test is within its expiration date. If kit contents were left open too long, the strips could have absorbed moisture, which can affect test results. Be sure to always keep the ImmunoStrip vial tightly sealed between uses. The test line may be weak due to a low-expressing lot of transgenic sample.

"This ImmunoStrip® test is intended for grain, seed and leaf quality purposes to determine the presence or absence of the AAD-12 protein which confers 2,4-D herbicide-resistance in transgenic soybean seed and leaves containing the Enlist E3™ Soybean trait and cotton seed and leaves containing the Enlist™ Cotton trait."