



Leading the way to healthy crops.

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USER GUIDE: 2mEPSPS ImmunoStrip® Test
Catalog number: 64100

KIT INFORMATION

Intended Use

This ImmunoStrip test is intended for qualitative determination of 2mEPSPS in soybean. The test limit of detection is one (1) 2mEPSPS soybean in 200 non-transgenic soybeans (0.5 % LOD).

This ImmunoStrip does not cross-react with other transgenic proteins in soybeans including CP4 EPSPS, PAT/*pat*, Bt-Cry1Ac, Bt-Cry1F, AAD-12, HPPD, and DMO.

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** for single leaf testing. Do not use any other sample extract buffer.

Storage of Kit

ImmunoStrips should be stored refrigerated (2 - 8 °C) between uses and tightly sealed in the desiccated container at all times.

Kit contents (including buffer) should be warmed to room temperature (18 - 30 °C) prior to use.

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?

- Tap water (composite seed and single seed)/ SEB4 buffer (ACC 01958) (leaf)
- Micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- 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
Soybean	Composite Seed	1:6 (weight:vol – g:mL)	Tap water	20 g:120 mL tap water
Soybean	Single Seed	1 seed + 1.0 mL	Tap Water	1 seed + 1.0 mL tap water
Soybean	Single Leaf	1:20 (weight:vol – g:mL)	1X SEB4	0.2 g:4.0 mL 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 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. Remove the jar from the blender and tap to collect all the powder. Shake the jar to mix and check for any un-ground seed.
3. Dispense the appropriate amount of tap water into the jar containing the entire sample of ground seed, close the lid and shake the bottle for 25 to 30 seconds. Let the extract sit for a minimum of 5 minutes before testing with the ImmunoStrip. Transfer 400 µL of supernatant (top layer of liquid) to a 1.5 mL microtube for testing.

Single Seed

A variety of single seed extraction methods may be used, providing that seeds are adequately crushed, extracted with 1 mL of 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:

- Seeds may be placed in the wells of a 48 testwell microtiter plate. Using a seed crusher insert, thoroughly crush seeds with rubber mallet. Add 1.0 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.
- 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 and massaged with your fingers for at least 15 seconds prior to settling and 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.
- Leaf tissue may also be extracted by cutting two leaf punches 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® 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.**

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

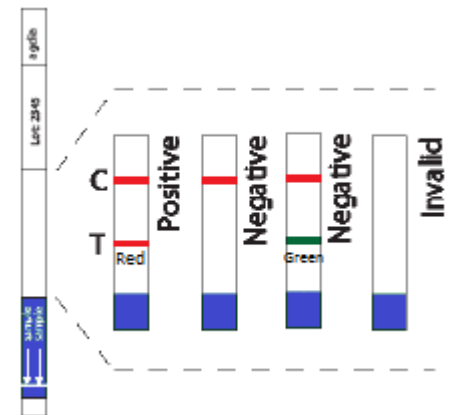
Single Seed and Single Leaf: Remove the ImmunoStrip after **5 minutes** of incubation with sample extract and interpret results.

If the sample is **positive** for the 2mEPSPS trait, a purple or red test line will appear. 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.

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.

Do not allow the ImmunoStrip to incubate in the extract for more than 10 minutes.

TROUBLESHOOTING



Control line did not develop.	This situation is generally caused by over-submergence of the test strip in the sample extract. Results in this situation 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.