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USER GUIDE: AAD-1 ImmunoStrip® Test
Catalog number 23500



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KIT INFORMATION

Intended Use

This ImmunoStrip® test is intended for grain, seed and leaf quality purposes to determine the presence or absence of the 2,4-D herbicide-resistance trait, *Enlist™* (AAD-1) in transgenic corn seed and leaf.

This is a single analyte ImmunoStrip® that has one specific test line. It has shown no cross-reaction with other transgenic proteins in corn leaf or seed including CP4 EPSPS, Bt-Cry1Ab/1Ac, Bt-Cry2A, Bt-Cry3A, mBt-Cry3A, Bt-Cry3Bb1, Bt-Cry34Ab1, Bt-Cry35Ab1, Bt-Cry1F, Bt-Cry9C, PAT/*pat*, GA21, GT21, CspB 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 **Water, 1X PBST or 1X SEB4** sample extraction buffer. See Page 2 for details on buffer selection. Do not use any other sample extraction buffer except those specified.

Storage of Kit

ImmunoStrips® should be stored refrigerated (4 °C) between uses and tightly sealed in the desiccated container at all times. Kit contents (including buffer) should be warmed to room temperature prior to use.

ImmunoStrips® Include

Size	Item
0050	ImmunoStrip® single strips, 50 count Instructions
0008	ImmunoComb® , 8 combs 12 strips per comb Instructions
0012	ImmunoComb® , 12 combs 8 strips per comb Instructions

What's required to perform the assay?

- PBST buffer (ACC 00501 or ACC 00115), recipe provided, or SEB4 buffer (ACC01958)
- Micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- Distilled or purified water
- Grinding equipment
 - Sample tube rack
 - Conical microtubes or conical microcentrifuge tubes (ACC 00340)
 - Pliers
 - Mesh sample bags (ACC 00930) and rubber mallet
 - Weigh paper
 - Golf Tee or disposable pestle

PREPARING THE SAMPLE

Single Seed

Seeds must be crushed and diluted in extraction buffer according to the ratios listed to the right:

Single seeds can be crushed with pliers or rubber mallet.

Determine the average weight of the seed and add the appropriate volume of buffer.

Tissue	Sample dilution with buffer (weight:vol – g:mL)	Example
SEED (PBST)	1:3	0.25 g : 0.75 mL buffer
SEED (SEB4)	1:3	0.25 g : 0.75 mL buffer

For example, take a single seed and fold in weigh paper. Crush with pliers and transfer crushed seed to a 1.5 mL conical microtube (or similar). Do not let seed come in direct contact with the pliers to avoid potential cross-contamination of samples. Add the appropriate amount of buffer, close the cap, and vigorously shake or vortex for 30 seconds. Allow the extract to settle for at least 1 minute before testing with the ImmunoStrip®.

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 buffer and mix the sample for at least 30 seconds. Allow the extract to settle for at least 1 minute before testing with the ImmunoStrip®.

Composite Seed Extraction

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

For composite seed samples (up to 100 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.

Tissue	Sample dilution in water (weight in grams : volume in mL)	Example
SEED	1:1.5	25 g seed: 37.5 mL water

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 one minute, remove the cap and transfer 350 µL of the supernatant (top layer of liquid) to a clean 1.5 mL microtube (or similar). Allow the extract in the micro tube to settle for at least 30 seconds before inserting the ImmunoStrip® for testing.

Single Leaf

Leaves must be macerated and diluted in extraction buffer according to the ratios listed to the right:

Leaf tissue can be tested in a 1.5 mL

conical microtube (or similar). See table above for number of leaf portions needed for the type of buffer being used.

Tissue	Sample dilution with buffer (weight:vol – g:mL)	Example
LEAF (PBST)	1:3	5 punches : 0.3 mL buffer
LEAF (SEB4)	1:5	3 punches : 0.3 mL buffer

Cutting leaf punches: Place the leaf directly over the top of an open 1.5 mL conical microtube (or similar). Press the cap down tightly to excise one circular leaf punch from tissue. Repeat until the designated number of punches has been obtained.

For example, take 5 leaf punches and place them at the bottom of a 1.5 mL conical microtube. Add 0.3 mL of 1X PBST buffer and macerate the leaf thoroughly (10-15 sec) with a golf tee or disposable pestle. For SEB4 buffer, follow the same procedure using the ratios listed in the table.

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 no more than ¼” or to the white line on the ImmunoStrip label.**

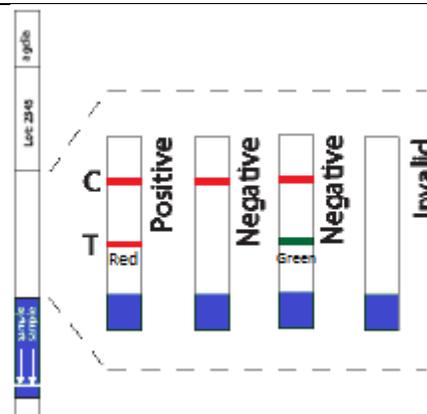
Single Seed/Single Leaf

Remove the ImmunoStrip® after **10 minutes** of incubation with the sample extract and interpret results. Positive results may be visible in as little as 5 minutes.

Composite Seed Testing

Remove the ImmunoStrip® after **10 minutes** of incubation with the sample extract and interpret results. Any red or pink signal in the test line area must be interpreted as positive.

Do not allow the ImmunoStrip® to incubate in the extract for more than the allotted time.



SAFETY

ImmunoStrips and sample extraction buffer are non-hazardous.

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. 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 and should be considered negative.										
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.										
PBST Buffer (1X)	Dissolve in distilled water to 1000 mL: <table style="width: 100%; border: none;"> <tbody> <tr> <td style="width: 70%;">Sodium chloride</td> <td style="text-align: right;">8.0 g</td> </tr> <tr> <td>Sodium phosphate, dibasic (anhydrous)</td> <td style="text-align: right;">1.15 g</td> </tr> <tr> <td>Potassium phosphate, monobasic (anhydrous)</td> <td style="text-align: right;">0.2 g</td> </tr> <tr> <td>Potassium chloride</td> <td style="text-align: right;">0.2 g</td> </tr> <tr> <td>Tween-20</td> <td style="text-align: right;">0.5 g</td> </tr> </tbody> </table> Adjust pH to 7.4	Sodium chloride	8.0 g	Sodium phosphate, dibasic (anhydrous)	1.15 g	Potassium phosphate, monobasic (anhydrous)	0.2 g	Potassium chloride	0.2 g	Tween-20	0.5 g
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Potassium chloride	0.2 g										
Tween-20	0.5 g										

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