



Leading the way to healthy crops.

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USER GUIDE: Bt-Cry1Ab/1Ac ImmunoStrip® Test
Catalog number: 06200

KIT INFORMATION

Intended Use

This ImmunoStrip test is intended for qualitative determination of Bt-Cry1Ab or Bt-Cry1Ac proteins in corn and cotton seed and leaf tissue.

This ImmunoStrip does not cross-react with other transgenic proteins in corn and cotton including CP4 EPSPS, 2mEPSPS, GT21, PAT/*pat*, PAT/*bar*, Bt-Cry1Ac, Bt-Cry1F, Bt-Cry2A, VIP3A, AAD12, Bt-Cry34Ab1, Bt-Cry35Ab1, Bt-Cry3Bb1, mBt-Cry3A, eCry3.1Ab, 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 **1X SEB4** for single seed and 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?

- SEB4 buffer (ACC 01958)
- 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
Corn and Cotton	Single Seed	1 seed + 1.0 mL	1X SEB4	1 seed + 1.0 mL 1X SEB4
Corn and Cotton	Single Leaf	1: 20 (weight: vol – g: mL)	1X SEB4	0.2 g: 4.0 mL 1X SEB4

PREPARING THE SAMPLE

Single Seed

A variety of single seed extraction methods may be used, providing that seeds are adequately crushed, extracted with 1 mL of 1X SEB4 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:

- 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. Agdia recommends removing the top half of the mesh bag prior to testing.
- Crush seed using a *mechanical* bead beater and extract the resulting seed particles using the sample concepts as above.
- Another method for single seed extraction, utilizing the strips in comb format, 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 1.0 mL of SEB4 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® comb.

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 – 0.3g. 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.

PERFORMING THE ASSAY

Insert the ImmunoStrip® into the microtube, or, if applicable, into the channel portion (no mesh) of the mesh bag.

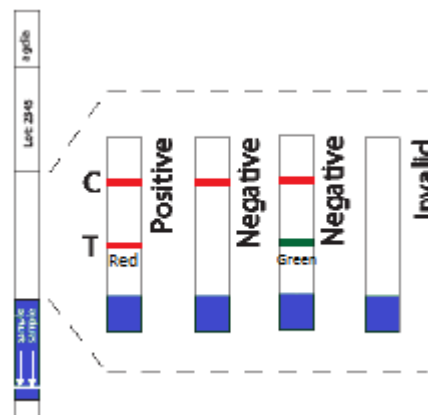
***Be sure to insert the "sample" end of the strip into the extract no more than ¼" or up to the white line on the ImmunoStrip.**

Remove the ImmunoStrip after **10 minutes** of incubation with the sample extract and interpret results. Positive results must be interpreted as any intensity of signal present in the test line area. 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.