

USER GUIDE: B3XF ImmunoStrip® Test

Catalog number: 53000

KIT INFORMATION

Intended Use

This ImmunoStrip is intended to detect Vip3A, DMO, CP4 EPSPS, Bt-Cry2Ab, and Bt-Cry1Ac in transgenic cotton seed.

This multi-analyte ImmunoStrip has shown no cross-reactivity with 2mEPSPS, AAD-12, Bt-Cry1F or Pat/bar proteins.

Individual lines are specific for Vip3A, DMO, CP4 EPSPS, Bt-Cry2Ab and Bt-Cry1Ac.

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 **SEB13** for single seed testing.

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 SEB13 buffer <http://docs.agdia.com/DataSheets.aspx>

ImmunoStrips Include

- ImmunoStrips
- User guide

What's required to perform the assay?

- SEB13 buffer (ACC 00993)
- Micropipette tips
- Graduated cylinder
- Scissors and pen
- Timer
- Extraction equipment
 - Mesh sample bags (ACC 00930) and rubber mallet (optional)
 - Weigh paper
 - 48 well microtiter plate
 - Seed crusher insert

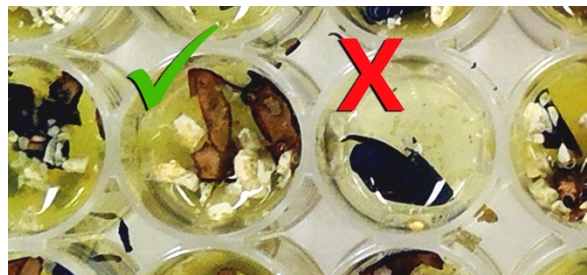
Validated Sample Dilution Ratios and Diluents

Host	Sample Type	Dilution Ratio	Diluent	Example
Cotton	Single Seed	1 seed + 0.75 mL	SEB13	1 seed + 0.75 mL SEB13

PREPARING THE SAMPLE

Single Seed

- Place single seeds in wells of a 48 testwell microtiter plate.
- Using a seed crusher insert or hydraulic press, thoroughly crush seeds.
- Add 0.75 mL of SEB13 buffer to each well.
- Shake on an orbital shaker at medium speed for 10 minutes. Allow extract to settle for 1 minute before testing with ImmunoStrip comb.
- Adequately crushed and extracted samples appear yellow to brown. Should the extract appear clear, sample may be an empty seed coat or sample was not properly extracted (See Figure). Re-crush and mix sample to ensure proper extraction before running ImmunoStrips. If seed material sticks to bottom of plate, gently mix with ImmunoStrip, while inserting it into well.



PERFORMING THE ASSAY

Insert the ImmunoStrip® into the channel portion (no mesh) of the mesh bag. When testing seeds in 48 well plates using combed ImmunoStrips®, add the combed strips vertically down the back of the wells and gently push to the bottom of the wells.

***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(s) after **10 minutes** of incubation with sample extract and interpret results.

Use the image to the right as a guide to determine results. If necessary, align the ImmunoStrip with the image to determine the exact positions of the test lines and the control line.

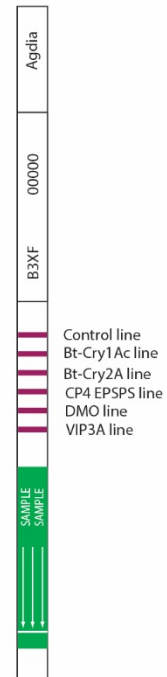
If the sample is positive for any of the proteins, a purple or red test line will appear. Test line intensity can vary depending on the available antigen in the sample. Test lines appearing as gray should be considered inconclusive and should be retested.

If the sample is negative for a protein, no test line will appear for that protein.

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.

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.



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 brown test line.	Brown 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.

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