Leading the way to healthy crops.

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USER GUIDE: GAT4621 ImmunoStrip® Test
Catalog number: 59000

KIT INFORMATION

Intended Use

This ImmunoStrip test is intended for qualitative determination of Optimum GLY® (GAT4621) in canola. The test limit of detection is nine GAT4621 canola seeds in 1000 non-transgenic canola seeds (0.9% LOD). This test kit has been validated and approved by Pioneer for detection of the Optimum GLY® trait in canola.

This ImmunoStrip does not cross-react with other transgenic proteins in canola seeds including NPTII, CP4 EPSPS or PAT/bar.

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 PBS** for composite seed testing 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 follow GLP procedures and refer to SDS for hazards associated with PBS.

ImmunoStrips Include

- ImmunoStrips
- User guide

What's required to perform the assay?

- PBS Powder (ACC00042)
- Micropipette tips
- Graduated cylinder
- Balance 1-500 gram
- Scissors and pen
- Timer
- Grinding equipment
  - Coffee or spice grinder
  - Sample tube rack
  - 50 mL conical centrifuge tube
  - 1.5 mL conical microtubes or conical microcentrifuge tubes (ACC 00340)
  - Mesh sample bags (ACC 00930)
  - Weigh paper
  - Golf Tee or disposable pestle

Validated Sample Dilution Ratios and Diluents

Host	Sample Type	Dilution Ratio	Diluent	Example
Canola	Composite Seed	1: 5 (weight: vol – g: mL)	1X PBS	5 g: 25 mL
Canola	Single Leaf	1: 20 (weight: vol – g: mL)	1X PBS	0.2 g: 4.0 mL

***Please see diluent preparation instructions on page 2.**

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 1:5 using 1X PBS. For this test, composite seed samples of 1,000 seeds can be extracted per sample. Agdia recommends using a KitchenAid coffee grinder with removeable grinding bowls (Model no. BCG211).

1. Place the weighed seed sample in a dry grinding bowl.
2. Grind the seed for 30 seconds. Remove the grinding bowl from the grinder and tap to collect all the powder. Shake the bowl to mix and check for any un-ground seed.
3. Transfer the ground seed to a 50mL conical centrifuge tube.
4. Dispense the appropriate amount of 1X PBS containing the entire sample of ground seed, close the lid and shake the tube for 25 to 30 seconds. Let the extract sit for a minimum of 5 minutes before testing with the ImmunoStrip. Transfer 500 µL of supernatant (top layer of liquid) to a 1.5 mL microtube for 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 PBS and allowed to settle for at least 1 minute before testing with the ImmunoStrip®.

Example method:

- 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 PBS is added and the leaf tissue is macerated by rubbing the pouch with a homogenizer or the end of a rounded blunt object. Remove the top half of the bag before testing.

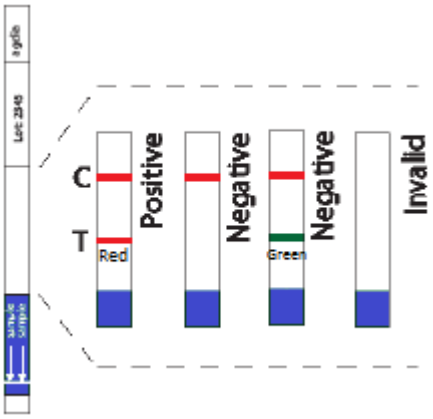
PERFORMING THE ASSAY

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.

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



PREPARING THE SAMPLE DILUENT

PBS diluents should be prepared by dissolving room temperature powders in distilled water in the approximate quantity needed per day. When preparing diluents, it is recommended to add the powder to a small amount of mixing distilled water then slowly bring the liquid up to the final volume. Once the final liquid volume has been added stir for 30 minutes or until dissolved.

Diluent Preparation Guide

Buffer	Usage Rate	Diluent	pH Range
1X PBS	9.55 grams/L	Distilled Water	7.2 to 7.6

***Using buffers older than one day is not supported but if you choose to, it is recommended that a preservative such as sodium azide be added at the rate of 0.2 grams per liter.**

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.

"Optimum GLY® is a Trademark of PIONEER."