



Strategic Diagnostics Inc.



Test and Be Sure

## Trait✓LL Test Kit

Part Number 7000043

Application for Bulk Canola Testing



### Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the PAT protein in canola bulk seed and grain samples. The Trait✓ LL test strip has a detection limit of one (1) LibertyLink® (PAT) canola seed in 50 non-LL canola seeds.

### Product Description

The Trait✓ LL Lateral Flow Test Kits detect the PAT protein produced by a gene derived from *Streptomyces hygroscopicus* or *S. viridochromogenes*. These genes have been incorporated into herbicide-resistant corn, cotton, canola and other crops including LibertyLink® brands from Bayer CropScience and other companies. The test will detect both the PAT proteins produced by both the *pat* and *bar* genes found in Liberty® resistant brands (LibertyLink®). The lateral flow strips and other kit components are sufficient to detect the presence or absence of the PAT protein in both field and laboratory environments. Different application protocols are required for leaf, seed and bulk grain detection. This product can screen LibertyLink® (InVigor) canola grain at specified levels.

### Principle of the Test

The assay uses a double antibody sandwich format. Antibodies specific to the PAT protein are coupled to a color reagent and incorporated into the lateral flow strip. When the lateral flow strip is placed in a small amount of an extract from plant tissue that contains PAT protein, binding occurs between the coupled antibody and the protein. A sandwich is formed with some, but not all the antibody that is coupled to the color reagent. The membrane contains two capture zones, one captures the bound PAT protein and the other captures color reagent. These capture zones display a reddish color when the sandwich and/or unreacted colored reagents are captured in the specific zones on the membrane. The presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.

### Contents of Kit

Description	Quantity
Trait✓ LL Lateral Flow Test Strips	2x50*
Sample Tubes (1.5 ml)	100*
Transfer pipettes	100*
User Guide	1

\* May contain more than 100 units.

### Materials Required but not Supplied

- Plastic bags, Ziploc, 8x12 cm, 4 mil thick (1350500)
- Rubber mallet
- Sample tube rack (6000023)
- Pipette, capable of delivering 1 mL

### Detection Limits

Screening grain at very low GM levels can be accomplished by using a sufficiently large sample size that test negative for the GM trait. Lateral flow strips can be used by testing multiple sub-samples, the size of which do not exceed the sensitivity of the strip test. **The Trait✓ LL strip test sensitivity is at least one LL canola seed in 50 seeds.**

### Principle of the Screening Application

This test protocol does not determine the exact percent of GMO canola seeds. It determines the probability that a sample contains greater or less than a specified threshold concentration. For LL canola, if one 50-seed sample is negative by the Trait✓ test strips, there is a 95% confidence that the sample contains less than 6% GMO content. Refer to the table below for other confidence levels with multiple 50-seed sub-samples.

**50 Seed Sub-Samples**  
(All Sub-Samples Must be Negative)

No. Sub-Samples of 50 Seeds	Percent GM using Sub-Sample Sizes of 50 Seeds at Five Different Confidence Levels				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
<b>1</b>	1.19	2.77	4.60	6.00	9.20
<b>2</b>	0.69	1.40	2.20	3.00	5.00
<b>3</b>	0.46	0.92	1.53	2.00	3.00
<b>4</b>	0.35	0.69	1.15	1.50	2.30
<b>5</b>	0.28	0.56	0.92	1.20	1.80
<b>6</b>	0.23	0.46	0.77	1.00	1.50

**Preparation and Storage of Reagents**

The Trait✓ Test Kits should be stored at room temperature. The Trait✓ Strips used in this kit must be kept in the canister with the desiccant. The moisture indicator card must be blue in color. Storage conditions higher than room temperature may adversely affect performance.

**Sampling**

The samples used for the Trait✓ Test Kits can be sub-samples of those “representative samples” collected from trucks, railcars, barges, etc. for other tests. The size of the sub-samples to be used for the Trait✓ tests will depend on the percent GMO screening level desired and an acceptable level of risk that the GMO level is close to the screening level.

*Note: It is assumed that the samples collected are representative of the contents of the truck or container and are sufficiently mixed to contain a random distribution of the sample contents.*

**Sample Preparation: Weigh the Sample**

The statistical sampling plan is dependent on the number of canola seeds used. However, it is more practical for routine testing to weigh canola seeds instead of counting to obtain the desired number of seeds. The average weight of canola seeds depends on the variety of canola and environmental conditions.

It is recommended that the weight-to-canola seed ratio for each variety be determined as follows.

1. Count 100 seeds of the variety to be tested.
2. Weigh the 100 seeds to the nearest 0.01 gram.
3. Divide the weight of the canola seeds by 100 to get the average grams per seed.
4. Multiply this average weight by the desired number of canola seeds in the sub-samples to determine the weight for the sub-samples.
5. Construct a weight-to-canola seed ratio table for each variety for the different sub-sample sizes to be used.

**Example:** One hundred (100) canola seeds of Variety A weigh 0.35 grams. Each canola seed then weighs 0.0035 grams. Multiply the 0.0035-gram per canola seed times the number of seeds in each sample size. This average weight is then used to obtain the number of seeds for this canola variety

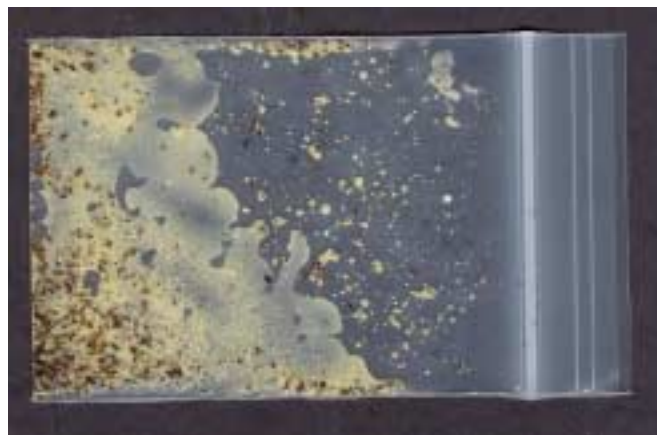
**Sample Preparation**

The canola sample is ground in the Ziploc plastic bag and then extracted with water directly in the bag. The sample preparation is important for the proper function of the test, especially the ratio of water to the weight of the canola sample.

1. Weigh 50-seed sub-samples from each truck or container.
2. Place each sub-sample in a clean, **dry** plastic bag
3. Place the bag on a hard surface and grind the seed dry by pounding the bag with the rubber mallet (or pestle) until all the seeds are broken. *Note: the seed coats will not be fully ground.*
4. Add 1.0 mL of water and mix the seed sample with the mallet until homogenous slurry is formed (approx. 1 min.). See example below.

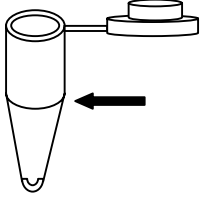
**Note:** The sample will have a “thick” consistency but should contain some free liquid after a short settling time. **There should be no whole seeds remaining.** Alternative methods of grinding the seed may also be used as long as all seeds are broken open and relatively homogeneous slurry is formed.

5. Use this free liquid as sample in the **Test Procedure**.



### Test Procedure

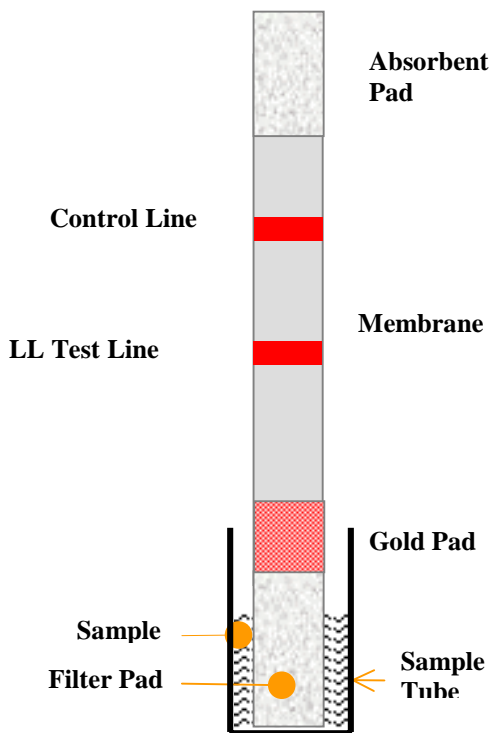
1. Transfer 0.5 mL of the liquid from the sample prepared above into a sample tube using the transfer pipette provided.



The sample tube has a 0.5-mL indicator at the top of the tapered section.

2. Place a Trait✓ LL Test Strip into the sample tube.
3. Let sit for 10 minutes.
4. The appearance of **one line** (control) on the strip indicates a **negative** result.
5. The appearance of **two lines** on the strip indicates a **positive** result.

### Illustration of Test Strip

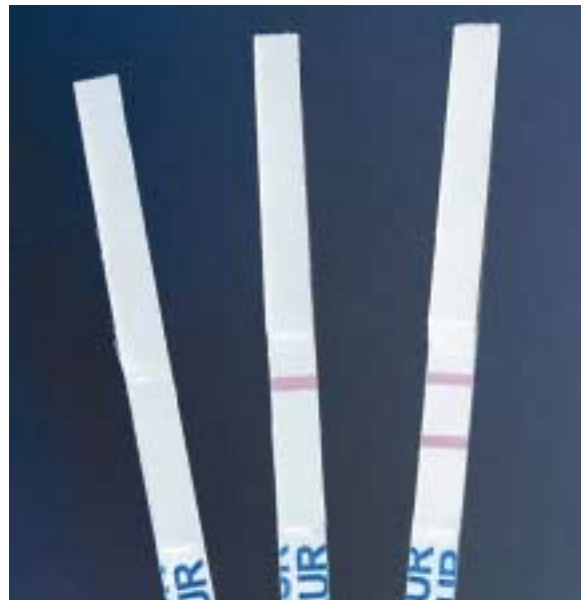


### Interpreting the Lateral Flow Strip Test

Check the result window at ten (10) minutes after inserting the strip. At least one line, the Control Line, should always develop approximately one (1) cm down from the Absorbent Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result. If the test strip displays two (2) red lines, the test is complete and the sample is positive for LL canola. If at 10-15 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for LL canola. If no control line develops, the result is inconclusive and need to be repeated.

**Note:** *Test strip results should be interpreted after 10 minutes. Test strips interpreted after 15 minutes are invalid.*

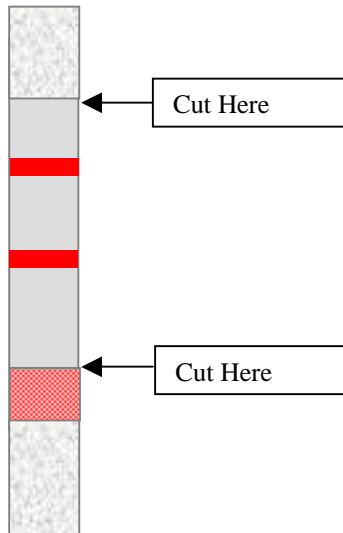
### Illustration of Positive and Negative Results



Example of an unreacted, negative (1-line) and positive (2-lines) test strip

**Archiving Test Strips**

If it is desired to archive test strip results, cut off the bottom and top strip pads as illustrated below within one (1) hour of test completion.



**For Technical Service call:**

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