



Trait✓ LL Lateral Flow Test User Guide

Trait✓ LL Corn Grain Test Kit (100-Test)
Part Number 7000043

Product Description

The Trait✓ LL Corn Grain Test Kit detects the PAT proteins produced by genes derived from *Streptomyces hygroscopicus* or *S. viridochromogenes*. These genes have been incorporated into herbicide-resistant corn, canola and other crops including LibertyLink® brands from Bayer CropScience and other companies. The test will detect both the PAT/pat and PAT/bar proteins found in Liberty® resistant brands (LibertyLink®, StarLink®, Bt-Xtra™, etc.). The intended uses of the kits include the qualitative (yes/no) determination of the PAT proteins in corn grain and other tissues. The lateral flow strips are sufficient to detect the presence or absence of the PAT proteins in up to 100 samples in both field and laboratory environments. Different crop tissue samples may require somewhat different testing protocols.

Principle of the Assay

The assay uses a double antibody sandwich format. Antibodies specific to the PAT proteins 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(s), binding occurs between the coupled antibody and the protein(s). A sandwich is formed with some, but not all, of the antibody that is coupled to the color reagent. The membrane contains two capture zones, one captures the bound PAT protein(s) 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*
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* May contain more than 100 units.

Materials Required but not Supplied

Laboratory grade blender (Waring Model 31BL91 recommended: (P/N 6000022)
Waring adapter for “Mason-type” jars (P/N 6000021)
Blender blade: custom (P/N 6000040)
Blender jar. “Mason” 4 oz (P/N 6000033)
Graduated cylinder, 50 ml (P/N 6000036)
Blender Shield (P/N 6000037)

Caution: Do not use larger jars than 4 oz. A shield should be used over the blender jars while grinding. Safety glasses should be used.

Preparation and Storage of Reagents

The Trait✓ LL Corn Grain Test Kit should be stored at room temperature. The Trait✓ LL Test 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✓ LL Corn Grain Test Kit 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✓ LL test will depend on the percent screening level desired and an acceptable level of risk that the LL level is close to the screening level. The number and size of the sub-samples will be discussed in more detail

in the **Principle of the Screening Application** section.

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: Weighing the Sample

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The statistical sampling plan (see **Principle of the Screening Application**) is dependent on the number of corn kernels used. However, it is more practical for routine testing to weigh corn kernels instead of counting to obtain the desired number of kernels. The average weight of corn kernels depends on the variety of corn and environmental conditions.

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

1. Count 100 kernels of the variety to be tested.
2. Weigh the 100 kernels to the nearest 0.01 gram.
3. Divide the weight of the corn kernels by 100 to get the average grams per kernel.
4. Multiply this average weight by the desired number of corn kernels in the sub-samples (selected in **Tables C, D or E**) to determine the weight for the sub-samples.
5. Construct a weight-to-corn kernel ratio table for each variety for the different sub-sample sizes to be used.

Example: One hundred (100) corn kernels of Variety X weigh 25.00 grams. Each corn kernel then weighs 0.25 grams. Multiply the 0.25-gram per corn kernel times the number of corn kernels in each sample size to get the following table.

Example: Weight-to-Corn Kernel Ratio Table

	Gram/Sample of		
No. Corn Kernels (a)	40	60	70
Sample Weight (g)	10.0	15.0	17.5

(a) From Table C

This average weight is then used to obtain the number of corn kernels for this corn variety for routine testing.

Sample Preparation: Processing the Sample

The corn sample is ground and then extracted with water in a glass “Mason”-type jar. The sample preparation is important for the proper function of the test, especially the ratio of water to the weight of the corn sample. The volume of water in milliliters (ml) should be close to 1.25 times the weight of corn sample in grams (g).

Sample Weight (g) X 1.25 = Water Volume (ml)

The processing parameters were determined using the laboratory grade Waring Model 31BL91 food processor with the standard blade (see **Materials Required but not Supplied**). Other food processors may require different parameters.

1. Weigh sub-samples from each truck or container.
2. Place each sub-sample in a clean, **dry** 4 oz. “Mason” jar.
3. Attach the jar adapter and clean, **dry** cutting blades.
4. Place the jar onto the food processor, place a shield over the jar and grind the sub-sample on high speed for **20 seconds**.

Caution: *It is recommended to shield the jars during grinding with a “tri-cornered” 1-liter plastic beaker (P/N 6000037).*

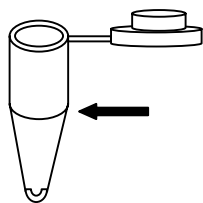
5. Remove the adapter and cutting blades.
6. Add the prescribed volume of water (see above) to the ground corn in the jar, place a lid on the jar and shake the jar until all the ground corn is well wetted (about 10-20 sec.).

Note: The sample will have a “thick” consistency but should contain some free liquid after a short settling time. **There should be no whole kernels remaining.**

7. 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.

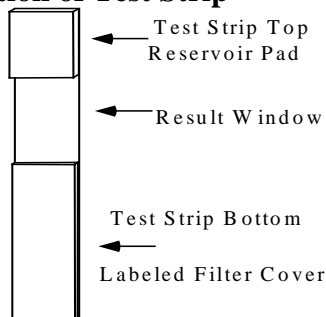


The sample tube has a 0.5-mL indicator at the top of the tapered section.

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

Interpreting the Lateral Flow Strip Test

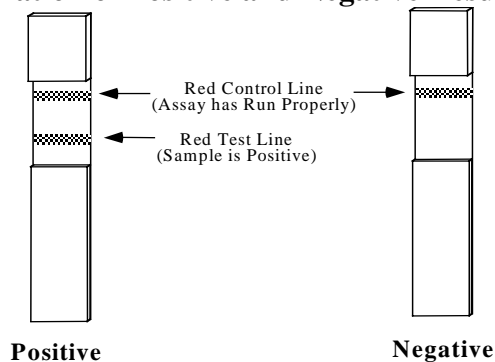
Illustration of Test Strip



Check the result window at five (5) minutes after inserting the strip. At least one line, the Control Line, should always develop approximately one (1) cm down from the Reservoir 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 corn. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for LL corn.

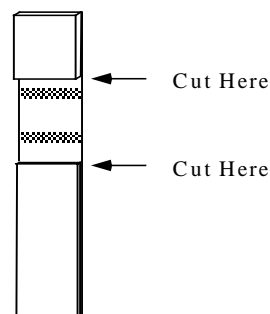
Note: Test strip results should be interpreted after 5 minutes. Test strips interpreted after 60 minutes are invalid.

Illustration of Positive and Negative Results



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.



Equipment Cleaning and Drying

Caution: It is important to clean and dry the jars and cutting blades between samples.

1. The “Mason” jar should be emptied, rinsed thoroughly with water and completely dried with a paper towel between uses.
2. The cutting blades for the blender should be rinsed with water until **all ground corn** is removed, washed using standard household liquid soap, rinsed well and carefully dried. If available, spraying or rinsing with methanol or isopropyl (rubbing) alcohol will assist drying.

Principle of the Screening Application

The Trait✓ LL test strip provides a yes/no answer for the presence or absence of Liberty-resistant corn kernels in a given sample. Testing two statistically selected sub-samples allows an estimate of the percent of Liberty-resistant corn kernels. The test results provide information

about the probability of the percent Liberty-resistant corn in the sample.

Note: The test protocol does not determine the exact percent of Liberty-resistant corn kernels. It determines the probability that a sample contains greater than a specified threshold concentration.

The statistical model used for this application is based on the Poisson Probability Distribution which provides good approximations to binomial (yes/no) probabilities when the number of items tested (i.e. corn kernels) is large but the probability of a positive result is expected to be small (i.e. low level of Liberty-resistant corn kernels). Using this Distribution, it is possible to determine the probability of having zero Liberty-resistant corn kernels in a random sample of a given number of corn kernels at a given percent Liberty-resistant corn. For example, a random sub-sample of 100 corn kernels selected from a larger population containing one- percent Liberty-resistant corn has a 36.8% probability of containing no Liberty-resistant corn kernels. The probability of a 75-corn kernel sub-sample (at one percent Liberty-resistant) containing zero Liberty-resistant corn kernels is 47.2%.

Screening at Very Low GM Levels

Screening grain at very low GM levels can be accomplished by using a sufficiently large sample size that tests 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 kernel in 70.**

The following tables provide information at five confidence levels with the use of multiple samples of 40 kernels 60 kernels or 70 kernels each. The tables provide the maximum percent GM levels that would be expected in the sample if all test-samples provide negative results. Either table can be used depending on the desired screening level and how the samples will be processed.

**Table C: 40 Seed Sub-Samples
(All Sub-Samples Must be Negative)**

No. Sub-Samples of 40 Seeds Each	Percent GM using Sub-Sample Sizes of 40 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	1.7	3.4	5.6	7.2	10.9
2	0.86	1.7	2.8	3.7	5.6
3	0.58	1.1	1.9	2.5	3.7
4	0.43	0.86	1.4	1.9	2.8
5	0.35	0.69	1.1	1.5	2.3
6	0.29	0.58	0.95	1.2	1.9
7	0.25	0.49	0.82	1.1	1.6
8	0.22	0.43	0.72	0.93	1.4

**Table D: 60 Seed Sub-Samples
(All Sub-Samples Must be Negative)**

No. Sub-Samples of 60 Seeds Each	Percent GM using Sub-Sample Sizes of 60 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	1.1	2.3	3.8	4.9	7.4
2	0.58	1.1	1.9	2.5	3.8
3	0.38	0.77	1.3	1.7	2.5
4	0.29	0.58	0.95	1.2	1.9
5	0.23	0.46	0.76	0.99	1.5
6	0.19	0.38	0.64	0.83	1.3
7	0.16	0.33	0.55	0.72	1.1
8	0.14	0.29	0.48	0.62	0.95

**Table E: 70 Seed Sub-Samples
(All Sub-Samples Must be Negative)**

No. Sub-Samples of 70 Seeds Each	Percent GM using Sub-Sample Sizes of 70 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	0.99	2.0	3.2	4.2	6.4
2	0.49	0.99	1.6	2.1	3.2
3	0.33	0.66	1.1	1.4	2.2
4	0.25	0.49	0.82	1.1	1.6
5	0.20	0.40	0.66	0.85	1.3
6	0.16	0.33	0.55	0.71	1.1
7	0.14	0.28	0.47	0.61	0.94
8	0.12	0.25	0.41	0.53	0.82

Choice of Confidence Level

The choice of the confidence level (and resulting sub-sample size) depends on how the test result information is to be used. If the primary concern is to have a very high confidence that the sample is below a certain GM screening level, then a higher confidence level and sample size is desired. However, this approach will “fail” some percentage of samples that are, in fact, below the screening level but somewhat close to it. The higher the confidence level chosen, the higher this failure rate will be.

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