



Strategic Diagnostics Inc.



Test and Be Sure

Seed✓LL Test Strips

Part Number 300008

Bulk Seed Testing



Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the PAT (LibertyLink®) protein in non-GM or conventional cottonseed samples. The Seed✓ LL Test Strip has a detection limit of one LL cottonseed in 1000 non-LL cottonseeds when used for seed purity testing of non-transgenic varieties (adventitious presence). When combined with a statistical sampling plan, test results can provide a level of confidence that a cottonseed sample contains LL cottonseed below certain percentages.

Product Description

The Seed✓ LL Test Kit detects the PAT protein produced by a *pat/bar* gene, derived from *Streptomyces hygroscopicus* or *S. viridochromogenes*. These genes have been incorporated into herbicide-resistant cotton, corn, canola and other crops including LibertyLink® brands from Bayer CropScience and other companies.. The lateral flow test strip has been optimized to easily detect the PAT protein expressed in bulk seed samples. The lateral flow strips in this package are sufficient to detect the presence or absence of the PAT protein in up to 50 bulk cottonseed samples.

Principle of the Assay

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

Seed✓ LL Test Strips

Quantity

50

Storage and Preparation of Reagents

The Trait✓ sample extraction buffer for this kit is shipped as a concentrate. Follow the procedure below to prepare the sample extraction buffer.

1. Pour the contents of a one (1) liter bottle of Trait✓ Sample Buffer Concentrate (P/N 7000006) into an 8-liter carboy or other suitable container.
2. Add four (4) liters of water to the sample buffer. Tap water may be used.
3. Mix well and label as Trait✓ Sample Buffer. Label buffer expiration as thirty (30) days from date of preparation.

The Seed✓LL Test Strips and Trait✓ Sample Buffer can be stored at room temperature or refrigerated. Do not freeze. Once opened, the Seed✓ LL lateral flow test strips must be stored in the closed canister with the indicating moisture card. The moisture-indicating card must be blue in color. If the moisture-indicating card is pink, contact SDI Technical Service. Storage conditions higher than room temperature may adversely affect performance

Materials Required but not Supplied for Bulk Cottonseed Testing:

Trait✓ Sample Buffer (7000006)
 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” 8 oz (P/N 6000034)
 Blender jar. “Mason” 16 oz (P/N 6000035)
 Graduated cylinder, 50 ml (P/N 6000036)
 Carboy, 8-liter (P/N 6000031)
 Blender Shield (P/N 6000037)
 User’s Guide (3090038)

Caution: A shield should be used over the blender jars while grinding. Safety glasses should used.

Purpose

The Seed✓ LL Test Kit has been designed to screen conventional bulk cottonseed samples for the presence of the PAT protein. Sample preparation procedures are outlined below. Please refer to the instructions to be sure that all of the necessary materials are available prior to testing.

Sample Preparation: Weighing the Sample

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

It is recommended that the weight-to-cottonseed 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 cottonseed by 100 to get the average grams per seed.
4. Multiply this average weight by the desired number of cottonseed in the sub-samples to determine the weight for the sub-samples.
5. Construct a weight-to-cottonseed ratio table for each variety for the different sub-sample sizes to be used.

Example: One hundred (100) cottonseeds of Variety X weigh 9.00 grams. Each cottonseed then weighs 0.09 grams. Multiply the 0.09-gram per cottonseed times the number of cottonseeds in each sample size to get the following table.

Table A: Example: Weight-to-Seed Ratio

No. Cottonseeds	Grams per Sample of X			
	100	200	500	1000
Sample Weight (g)	9.0	18.0	45.0	90.0

This average weight is then used to obtain the number of cottonseeds for this cotton variety.

Sample Preparation: Processing the Sample

The cottonseed is ground and then extracted with buffer in a glass “Mason”-type jar. The sample preparation is important for the proper function of the test, especially the ratio of buffer to the weight of the cottonseed. The volume of buffer in milliliters (mL) should be 3 times the weight of cottonseed in grams (g).

The size of “Mason” jar required and the grinding time depends on the sample size to be analyzed. **Table B** lists those parameters.

Table B: Parameters for Preparing Samples

Number of Seeds in Sample	Jar Size (oz.)	Grind Time (sec)
200-500	8	15-20
>500	16	15-20

The processing parameters were determined using the laboratory grade Waring Model 31BL91 food processor with the standard blade. Other food processors may require different parameters.

Sample Processing

1. Weigh sub-samples from each lot or container.
2. Place each sub-sample in a clean, **dry** “Mason” jar of the appropriate size. See **Table B**.
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 the time indicated in **Table B**.

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 volume of Trait✓ Sample Buffer (see below) to the ground cottonseed 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.).

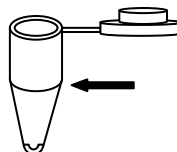
Sample Weight (g) X 3.0 = Buffer Volume (mL)

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.**

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

Test Procedure

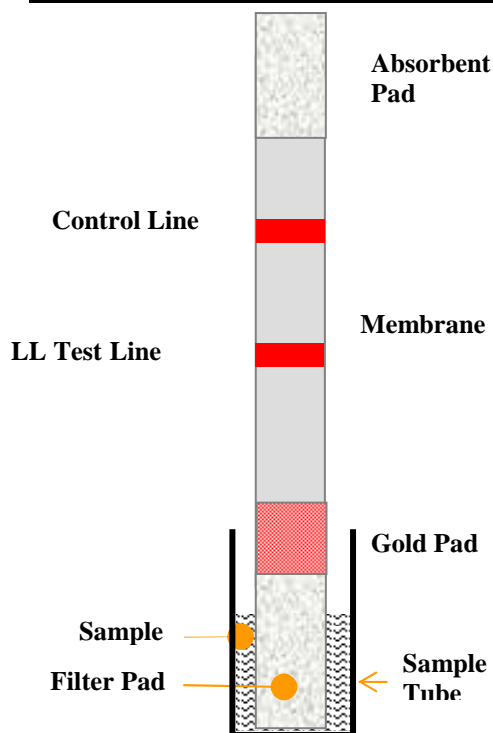
1. Transfer the liquid from the sample prepared to a sample tube by squeezing the bulb of the transfer pipette and inserting it in the free liquid in the top of the sample jar.
2. Release the bulb to pull up the sample. Add the sample from the pipette to the sample tube by squeezing the bulb. This should be approximately 0.5 mL.



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

3. Place one Seed✓ LL Test Strip into the sample tube. Let sit for 5 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 a Lateral Flow Strip



Note: Some leaf samples may produce a light brown or greenish-yellow color at the test line. **This is a negative result.** A positive result produces a distinct reddish color.

Interpreting the Lateral Flow Strip Test

Check the test strips 5 minutes after inserting the strip or comb. 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 strip is functioning properly. A red line appearing below the Control Line is the LL Test Line and indicates a positive result for PAT protein. If the test strip displays two (2) red lines, the test is complete and the sample is positive for LL trait. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for LL transgenic traits. If no control line develops, the result is inconclusive and need to be repeated.

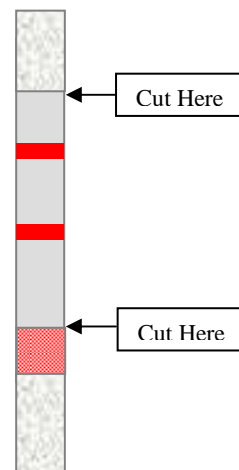
Illustration of Positive and Negative Results



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.



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 cottonseed** 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

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 Seed✓ LL strip test sensitivity is at least one cottonseed in 1000.**

The Seed✓ LL Test Strip provides a yes/no answer for the presence or absence of LL cottonseed in a given sample. Testing multiple statistically selected sub-samples allows an estimate of the percent of LL cottonseed. The test results provide information about the probability of the percent LL cottonseed in the sample.

Note: The test protocol does not determine the exact percent of LL cottonseed. It determines the probability that a sample contains greater or less than a specified threshold concentration.

Statistical Interpretation

The following tables provide information at five confidence levels with the use of multiple samples of 200, 400 and 1000 cottonseeds 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: 200 Seed Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 200 Seeds Each	Percent GM using Sub-Sample Sizes of 200 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	0.35	0.70	1.1	1.5	2.5
2	0.17	0.35	0.58	0.75	1.2
3	0.12	0.23	0.39	0.50	0.78
4	0.087	0.18	0.29	0.38	0.58
5	0.070	0.14	0.24	0.30	0.47
6	0.058	0.12	0.20	0.25	0.39
7	0.050	0.10	0.18	0.22	0.35
8	0.045	0.085	0.15	0.19	0.29

Table D: 400 Seed Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 400 Seeds Each	Percent GM using Sub-Sample Sizes of 400 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	0.17	0.35	0.58	0.75	1.2
2	0.087	0.18	0.29	0.38	0.58
3	0.058	0.12	0.20	0.25	0.39
4	0.045	0.085	0.15	0.19	0.29
5	0.035	0.070	0.12	0.15	0.25
6	0.029	0.058	0.10	0.13	0.20
7	0.025	0.050	0.085	0.11	0.18
8	0.022	0.044	0.075	0.10	0.15

Table E 1000 Seed Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 1000 Seeds Each	Percent GM using Sub-Sample Sizes of 1000 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	0.070	0.139	0.231	0.300	0.461
2	0.035	0.070	0.116	0.150	0.231
3	0.024	0.047	0.077	0.100	0.155
4	0.018	0.035	0.058	0.075	0.116
5	0.014	0.028	0.047	0.060	0.093
6	0.012	0.024	0.039	0.050	0.077
7	0.010	0.020	0.033	0.043	0.066
8	0.009	0.018	0.029	0.038	0.058

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For Technical Service call:

Strategic Diagnostics Inc.
111 Pencader Drive
Newark, DE 19702

Phone: 800-544-8881
Fax: 302-456-6782
E-mail: techservice@sdix.com

