Strategic Diagnostics Inc. **Trait✓**

Trait✓ Bt1/LL Lateral Flow Test User Guide

Trait✓ Bt1/LL Corn Grain Test Kit (100-Test)
Part Number 7000044

Product Description

The Trait✓ Bt1/LL Corn Grain Test Kit and this User Guide are designed to screen for both Bt and LibertyLink® corn together. For testing Bt corn alone, the Trait✓ Bt1 Corn Grain Test Kit (7000025) and corresponding User Guide (3090012) are recommended. For testing LibertyLink® corn alone, the Trait✓ LL Corn Test Kit (7000043) and corresponding User Guide (3090026) are recommended.

The Trait✓ Bt1/LL Lateral Flow Test Kits detect the Cry1Ab protein produced by a gene derived from *Bacillus thuringiensis (Bt)* and the PAT proteins produced by genes derived from *Streptomyces hygroscopicus* or *S. viridochromogenes*. These genes has been incorporated into insect-resistant corn including YieldGard® brands from Monsanto and Novartis, KnockOut® from Novartis and NatureGard® from Mycogen Seeds and herbicide-resistant corn including Liberty Link® brands from Bayer CropScience, Pioneer and others. The intended use of the kit is the qualitative (yes/no) determination of the Cry1Ab and PAT proteins in corn grain samples. Different application protocols are required for leaf, seed and bulk grain detection.

Principle of the Assay

The assay uses a double antibody sandwich format. Antibodies specific to the Cry1Ab or 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 Cry1Ab or 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 Cry1Ab or 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✓ Bt1 Lateral Flow Test Strips	2x50
Trait✓ LL Lateral Flow Test Strips	2x50
Trait✓ Sample Buffer Concentrate (liter)	1
Sample tubes	100*
Transfer pipettes	100*
User Guide	1

* 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)
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 used.

Preparation and Storage of Reagents

The Trait✓ Bt1 and 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✓ Bt1/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✓ Bt1 and LL tests will depend on the percent GM screening level and an acceptable level of risk that the GM 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

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.

Table A is a guideline for the average weight of corn seed kernels in several size ranges.

Table A: Average Weight of Seeds (derived from data from Iowa State University)

Seed Size	Ave. Grams / Seed
Very Small	0.188
Small	0.219
Medium	0.257
Large	0.316

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

1. Count 70 kernels of the variety to be tested.
2. Weigh the 70 kernels to the nearest 0.01 gram.
3. Divide the weight of the corn kernels by 70 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 or D**) 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: Seventy (70) corn kernels of Variety X weigh 17.50 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

	Grams per Sample		
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)

1. Weigh two 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 (see **Materials Required but not Supplied** for proper blades)

4. Place the jar onto the food processor, place a shield over the jar and grind the sub-sample for 10-15 seconds on high speed.

Caution: It is recommended to shield the jars during grinding using a Fisher "tri-cornered" 1-liter plastic beaker (02-593-50 F).

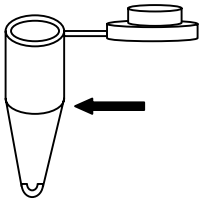
5. Remove the adapter and cutting blades.
6. Add the prescribed volume of water to the ground corn kernels in the jar (see **Table B**), place a lid on the jar and shake the jar until all the ground corn is well wetted (about 10-20 sec.).

Note: The sample may have a "thick" consistency with little or no free liquid. There should be no whole kernels remaining.

7. Use this sample immediately 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.

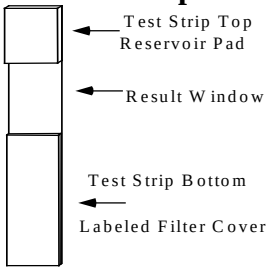


The sample tube has a 0.5-mL indicator at the top of the tapered section.

2. Place a Trait✓ Bt1 Test Strip and a Trait✓ LL Test Strip into each 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

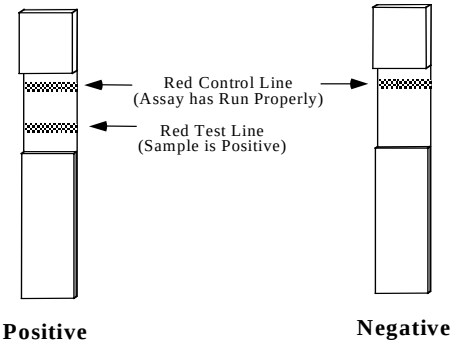
Illustration of Test Strip



Check the result window frequently 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 Bt or Liberty-resistant corn. If at about 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Bt or Liberty-resistant corn.

Note: If the sample contains high levels of Bt or Liberty resistant corn the control line may be faint and the test line very strong.

Illustration of Positive and Negative Results



Note: It is important to clean and dry the jars and cutting blades between samples. Caution should be taken during handling of the household bleach.

Equipment Cleaning and Drying

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 until all solid corn is removed, wiped clean, sprayed or rinsed with household bleach and dried with a paper towel between uses.

Principle of the Screening Application

The Trait✓ Bt1 and Trait✓ LL test strips provide a yes/no answer for the presence or absence of Cry1Ab Bt or PAT Liberty-resistant corn kernels in a given sample. Testing two statistically selected sub-samples allows an estimate of the

percent of GM corn kernels. The test results provide information about the probability of the percent GM corn in the sample.

Note: The test protocol does not determine the exact percent of GM corn kernels. It determines the probability that a sample contains less than a specified threshold (screening) 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 GM corn kernels). Using this Distribution, it is possible to determine the probability of having zero GM corn kernels in a random sample of a given number of corn kernels at a given percent GM corn. For example, a random sub-sample of 100 corn kernels selected from a larger population containing one- percent GM corn has a 36.8% probability of containing no GM corn kernels. The probability of a 50-corn kernel sub-sample (at one percent GM) containing no GM corn kernels is 60.7%.

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 and the sensitivity of the Trait✓ Bt1 strip test is at least one Bt kernel in 125. Therefore if both traits are evaluated in the sample, a 70-kernel maximum sample size can be used.**

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 (%)				
	50	**75**	**90**	**95**	**99**
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 (%)				
	50	**75**	**90**	**95**	**99**
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 (%)				
	50	**75**	**90**	**95**	**99**
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

Interpretation of Test Results

The screening results described below apply to YieldGard® brands (events MON810 and Bt11), KnockOut® and NatureGard® brands (Event 176), and to Liberty® resistant hybrids (events T25, DBT418, CBH-351, etc.

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 larger 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.

Lowering the sample size (and confidence level) will reduce the percentage of "good" samples that are rejected but somewhat increase the risk of a sample being above the screening level.

If a balance must be made between accepting as many "good" loads as possible and avoiding acceptance of too many samples above a certain level, a confidence level close to 50% is recommended.

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