Strategic Diagnostics Inc. **Trait✔**

Trait✔ Bt1 Lateral Flow Test User Guide

Trait✔ Bt1 *5-Minute* Corn Grain Test Kit (100-Test)
Part Number 7000025

Product Description

The Trait✔ Bt1 Lateral Flow Test Kits detect the Cry1Ab protein produced by a gene derived from *Bacillus thuringiensis (Bt)*. This gene has been incorporated into insect-resistant corn including YieldGard® brands from Monsanto and Novartis, KnockOut® from Novartis and NatureGard® from Mycogen. The intended use of the kit is the qualitative (yes/no) determination of the Cry1Ab protein in corn grain samples. The lateral flow strips and other kit components are sufficient to detect the presence or absence of the Cry1Ab protein in both field and laboratory environments. Different application protocols are required for leaf, seed and bulk grain detection. This product can screen for YieldGard® (MON810 and Bt11) in grain but can also detect KnockOut® and NatureGard (Event 176) at specified levels.

Principle of the Assay

The assay uses a double antibody sandwich format. Antibodies specific to the Cry1Ab 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 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 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
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; SDI P/N 6000022)
Waring adapter for "Mason-type" glass jars (6000021)
Blender jars (“Mason-type”; 4, 8, 16 and 32 oz.)
Sample tube rack (6000023)
Graduated cylinder, 250 ml (6000024)
Blender Shield (P/N 6000037)

Preparation and Storage of Reagents

The Trait✔ Bt1 Corn Grain *5-Minute* Test Kit should be stored at room temperature. The Trait✔ Bt1 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 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 test will depend on the percent Bt screening level and an acceptable level of risk that the Bt 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.

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.

Table A: Example: Weight-to-Kernel Ratio

	Grams per Sample of			
No. Corn Kernels (a)	40	50	70	100
Sample Weight (g)	**10.0**	**12.5**	**17.5**	**25.0**

(a) From Tables C, D and E.

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

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 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 Kernels in Sample	**Jar Size (oz.)**	**Grind Time (sec)**
25-125	4	10-20
125-250	8	15-25
250-500	16	20-35
>500	32	45-60

Note: Refer to the Application Procedure *Common Extraction Procedure to Detect Cry1Ab, Cry9C and CP4 EPSPS Proteins in Corn Grain* for interpretation of GMO content of samples greater than 100 kernels.

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** "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).

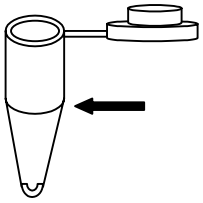
5. Remove the adapter and cutting blades.
6. Add the prescribed volume of water (see above) to the ground corn in the jar, place a lid on the jar and shake the jar until all the ground corn is well wetted (about 10-20 sec.).

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.

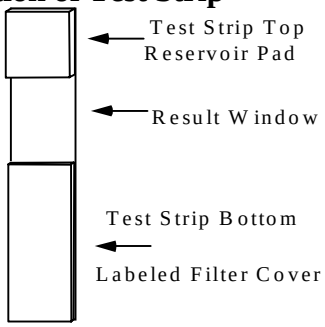


The sample tube has a 0.5-mL indicator at the top of the tapered section.

2. Place one Trait✔ Bt1 Test Strip into the sample tube. Let sit for 3-5 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

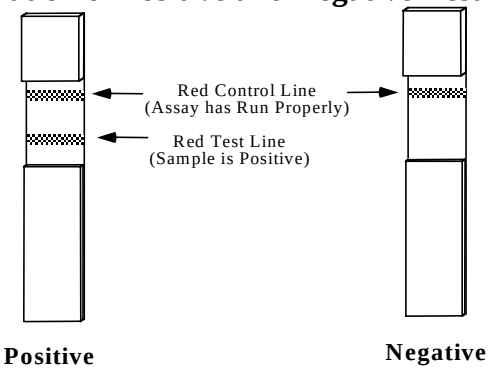
Illustration of Test Strip



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 Cry1Ab Bt corn. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Cry1Ab Bt corn.

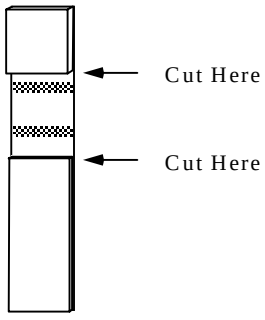
Note: ***Test strip results should be interpreted after 5 minutes. Test strips interpreted after 60 minutes are invalid.***

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✔ Bt1 Test Strip provides a yes/no answer for the presence or absence of Cry1Ab Bt corn in a given sample. Testing multiple statistically selected sub-samples allows an estimate of the percent of Cry1Ab corn. The test results provide information about the probability of the percent Bt corn in the sample.

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

The statistical model 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 Bt corn). This Distribution can determine the probability of having no Bt kernels in a random sample of a given number of kernels at a given percent Bt. For example, a random sub-sample of 100 corn kernels selected from a larger population containing one- percent Bt corn has a 36.8% probability of containing no Bt corn kernels. The probability of a 75-corn kernel sub-sample (at one percent Bt) containing zero Bt 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✔ Bt1 strip test sensitivity is at least one kernel in 100 for Bt11 varieties and one kernel in 50 for MON810 varieties.**

The following tables provide information at five confidence levels with the use of multiple samples of 50 kernels 70 kernels or 100 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: 50 Kernel Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 50 Kernels Each	Percent GM using Sub-Sample Sizes of 50 Kernels at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	1.39	2.77	4.6	6.0	9.2
2	0.69	1.39	2.2	3.0	5.0
3	0.46	0.92	1.5	2.0	3.1
4	0.35	0.69	1.2	1.5	2.3
5	0.28	0.56	0.92	1.2	1.8
6	0.23	0.46	0.77	1.0	1.5
7	0.20	0.40	0.66	0.86	1.3
8	0.17	0.35	0.58	0.75	1.1

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

No. Sub-Samples of 70 Kernels Each	Percent GM using Sub-Sample Sizes of 70 Kernels at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	1.00	2.00	3.30	4.30	6.60
2	0.50	0.99	1.65	2.15	3.30
3	0.33	0.66	1.43	1.65	2.20
4	0.25	0.50	0.83	1.07	1.65
5	0.20	0.40	0.66	0.86	1.32
6	0.17	0.33	0.55	0.72	1.10
7	0.14	0.28	0.47	0.61	0.94
8	0.12	0.25	0.41	0.54	0.83

Table E: 100 Kernel Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 100 Kernels Each	Percent GM using Sub-Sample Sizes of 100 Kernels at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	0.70	1.40	2.20	3.00	5.00
2	0.35	0.70	1.16	1.50	2.40
3	0.24	0.48	0.78	1.00	1.56
4	0.17	0.36	0.60	0.76	1.16
5	0.14	0.28	0.48	0.60	0.94
6	0.12	0.24	0.40	0.50	0.78
7	0.10	0.20	0.36	0.44	0.70
8	0.09	0.17	0.30	0.38	0.60

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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