Strategic Diagnostics Inc.

Test and Be Sure

Trait✓Cry3Bb Test Kit

Part Number 7000041

Bulk Grain Testing

Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the Cry3Bb1 protein in corn grain samples. The Trait✓ Cry3Bb test strip has been validated for detection limit of one (1) Cry3Bb YieldGard® Rootworm YieldGard® Plus, YieldGard VT Rootworm/RR2™, or YieldGard VT Triple™ corn kernel in 300 non-Cry3Bb kernels. This test does not differentiate between the Cry3Bb1 proteins produced in YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ corn hybrids.

Product Description

The Trait✓ Cry3Bb Lateral Flow Test Kits detect the Cry3Bb protein produced by a gene derived from *Bacillus thuringiensis (Bt)*. This gene has been incorporated into insect-resistant corn including YieldGard® Rootworm brands from Monsanto and other companies. . The lateral flow strips and other kit components are sufficient to detect the presence or absence of the Cry3Bb protein in both field and laboratory environments. Different application protocols are required for leaf, seed and bulk grain detection. This product can screen YieldGard® Rootworm (MON863), YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ (MON88017) corn hybrids at specified levels.

Principle of the Test

The assay uses a double antibody sandwich format. Antibodies specific to the Cry3Bb 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 Cry3Bb 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 Cry3Bb 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✓ Cry3Bb 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

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)
Methanol (laboratory grade)

Preparation and Storage of Reagents

The Trait✓ Cry3Bb Corn Grain Test Kit should be stored at room temperature. The Trait✓ Cry3Bb Test Strips used in this kit must be kept in the canister with the desiccant. The moisture indicator card must be blue in color. If the moisture indicator card is pink, please contact SDI Technical Services. Storage conditions higher than room temperature may adversely affect performance.

Sampling

The samples used for the Trait✓ Cry3Bb 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✓ Cry3Bb test will depend on the percent Cry3Bb screening level desired and an acceptable level of risk that the Cry3Bb 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 X			
No. Corn Kernels (a)	100	200	250	300
Sample Weight (g)	**25**	**50**	**62.5**	**75**

(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

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). 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)
25-100	4	10-20
1000-200	8	15-25
200-300	16	20-35

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.

Sample Processing

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**.

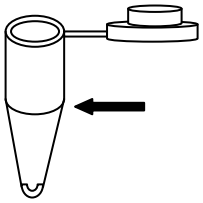
   Caution: It is recommended to shield the jars during grinding with a "tri-cornered" 1-liter plastic beaker (P/N 6000037).
5. Remove the adapter and cutting blades.
6. Add the volume of water (see below) to the ground corn in the jar, place a lid on the jar and shake the jar until all the ground corn is well wetted (about 10-20 sec.).

   Sample Weight (g) X 1.25 = Water 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 kernels remaining.**
7. Use this free liquid as sample in the **Test Procedure**.

Test Procedure

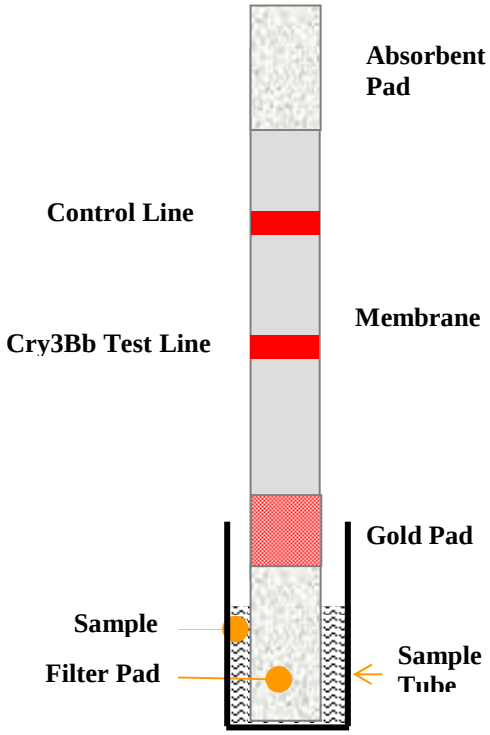
1. Transfer the liquid from the sample prepared to a sample tube by squeezing the bulb of the transfer pipette and inserting it in the free liquid in the top of the sample jar.
2. Release the bulb to pull up the sample. Add the sample from the pipette to the sample tube by squeezing the bulb. This should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



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

3. Place one Trait✓ Cry3Bb Test Strip into the sample tube. Let sit for 3-5 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 Test Strip



Interpreting the Lateral Flow Strip Test

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 Cry3Bb corn. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Cry3Bb corn. If no control line develops, the result is inconclusive and need to be repeated.

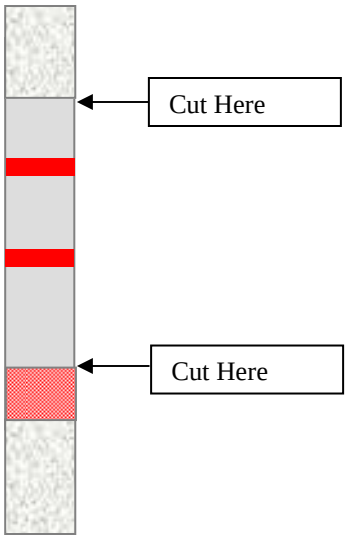
Note: ***Test strip results should be interpreted after 5 minutes. Test strips interpreted after 60 minutes are invalid.***

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.



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

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✓ Cry3Bb strip test sensitivity is at least one Cry3Bb kernel in 300.**

The Trait✓ Cry3Bb Test Strip provides a yes/no answer for the presence or absence of Cry3Bb corn in a given sample. Testing multiple statistically selected sub-samples allows an estimate of the percent of Cry3Bb corn. The test results provide information about the probability of the percent Cry3Bb corn in the sample.

Note: The test protocol does not determine the exact percent of Cry3Bb corn kernels. It determines the probability that a sample contains greater or less than a specified threshold concentration.

Statistical Interpretation

The following tables provide information at three confidence levels with the use of multiple samples of 100 kernels 200 kernels or 300 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: 100 Seed Sub-Samples (All Sub-Samples Must be Negative)

No. Sub-Samples of 100 Seeds Each	Percent GM using Sub-Sample Sizes of 100 Seeds at Five Different Confidence Levels (%)		
	90	**95**	**99**
1	2.31	3.00	4.70
2	1.16	1.50	2.31
3	0.77	1.00	1.53
4	0.58	0.75	1.16
5	0.47	0.60	0.93
6	0.39	0.50	0.78
7	0.330	0.430	0.660
8	0.288	0.375	0.575

Table D: 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 (%)		
	90	**95**	**99**
1	1.14	1.50	2.28
2	0.57	0.75	1.14
3	0.38	0.50	0.76
4	0.29	0.37	0.57
5	0.23	0.30	0.46
6	0.19	0.25	0.38
7	0.16	0.21	0.33
8	0.14	0.19	0.29

Table E: 300 Seed Sub-Samples (All Sub-Samples Must be Negative)

No. Sub-Samples of 300 Seeds Each	Percent GM using Sub-Sample Sizes of 300 Seeds at Five Different Confidence Levels (%)		
	90	**95**	**99**
1	0.76	0.99	1.52
2	0.38	0.50	0.76
3	0.26	0.33	0.51
4	0.19	0.25	0.38
5	0.15	0.20	0.31
6	0.13	0.17	0.26
7	0.11	0.14	0.22
8	0.10	0.12	0.19

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