



Strategic Diagnostics Inc.



Test and Be Sure

Seed ✓ Cry1F Test Strips

Part Number 3000016

Seed & Leaf Testing



Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the Cry1F protein in WideStrike® cotton leaf and cottonseed samples.

Product Description

The Seed ✓ Cry1F Test Kit detects the Cry1F delta endotoxin protein produced by a gene, derived from *Bacillus thuringiensis* (Bt), that is found in pre-commercial cotton varieties sold under the WideStrike® trade name. The lateral flow test strip has been optimized to easily detect the Cry1F protein expressed in the seed embryo but not detect the protein in a positive seed coat. The lateral flow strips in this package are sufficient to detect the presence or absence of the Cry1F protein in up to 50 cotton leaf or cottonseed samples.

Principle of the Assay

The assay uses a double antibody sandwich format. Antibodies specific to the Cry1F 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 Cry1F 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 Cry1F 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 ✓ Cry1F Test Strips

Quantity

50

The Seed ✓ Cry1F Test Strips & Combs and Trait ✓ Sample Buffer can be stored at room temperature or refrigerated. Do not freeze. Once opened the Seed ✓ Cry1F lateral flow test strips must be stored in the closed canister with the indicating desiccant card. The moisture-indicating desiccant card must be blue in color. If the moisture-indicating desiccant card is pink, contact SDI Technical Service. Storage conditions higher than room temperature may adversely affect performance

Materials Required but not Supplied for

Cottonseed Testing:

Trait ✓ Sample Buffer (7000006)
Sample tubes, 1.5 mL (3000411)
Weigh paper or waxed paper
Pliers for crushing seed
User Guide (3099938)

Purpose

The Seed ✓ Cry1F Test Kit has been designed to screen cottonseed and cotton leaf samples for the presence of the Cry1F 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.

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.

Single Cottonseed Sample Preparation

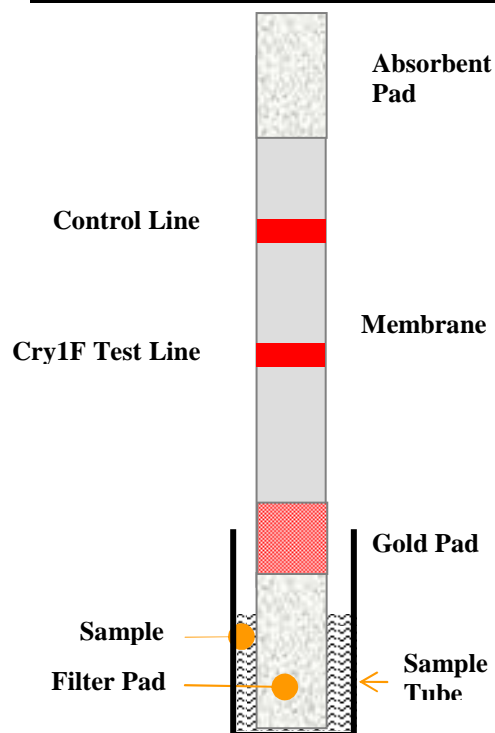
1. Place a cottonseed on a piece of weigh paper or waxed paper.
2. Fold the paper over the cottonseed and crack or smash into small pieces using pliers or other suitable means.
3. Put the cracked cottonseed into a 1.5-ml sample tube and add about 0.75 mL of Trait✓ Leaf & Seed Sample Buffer to the tube.
4. Let the tube stand for 3-5 minutes while shaking intermittently or stirring with the stirrer.
5. Insert the labeled Filter Cover of the Seed✓ Cry1F lateral flow test strip into the sample tube containing the cottonseed sample extract. The arrows on the filter cover should point into the tube. Allow the test strip to remain in the tube in an upright position for 5 minutes.
6. Proceed to *Interpreting the Lateral Flow Test Strip*

Leaf Sample Preparation

1. Fold a fresh cotton leaf twice and place the four thickness' between the body and cap of a 1.5 mL sample tube and snap the cap into place.
2. Open the cap and remove the excess leaf tissue from around the tube opening.
3. Push the leaf tissue into the tube with the stirrer. (This provides about 30-50 mg of cotton leaf tissue. The appropriate amount of tissue may be obtained by other means.)
4. Add about 0.5 mL (to the top of the tapered part of the tube) of Sample Buffer to the sample tube containing the leaf sample.
5. Macerate the cotton leaf material with a clean stirrer for about a minute.
6. Insert the labeled Filter Cover of the Seed✓ Cry1F lateral flow test strip into the sample tube containing the cotton leaf sample extract. The arrows on the filter cover should point into the tube. Allow the test strip to remain in the tube in an upright position for 5 minutes.
7. Proceed to *Interpreting the Lateral Flow Test Strip*.

Caution: The Seed✓ Cry1F test is sensitive. Be careful to not contaminate the leaf sample discs by touching them with your fingers, a stirrer or other material that may have touched another leaf sample.

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 Cry1F Test Line and indicates a positive result for Cry1F protein. If the test strip displays two (2) red lines, the test is complete and the sample is positive for Bt Cry1F traits. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Cry1F 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

Warranties and Liabilities

Strategic Diagnostics Inc. ("SDI") warrants the Products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal to the shorter of one year from date of shipment of the Product(s) or the expiration date marked on the Product packaging. Application protocols published by SDI are intended to be only guidelines for the Buyers of the Products. Each Buyer is expected to validate the applicability of each application protocol to in their individual applications. **SDI MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.**

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