



Strategic Diagnostics Inc.



Test and Be Sure

## Seed✓ B2R Test Strips & Combs

Part Number 7805000 & 7805100

Seed & Leaf Testing



### Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the Cry1Ac, Cry2Ab and/or CP4 EPSPS proteins in Bollgard® II/Roundup Ready® cotton leaf and cottonseed samples.

### Product Description

The Seed✓ B2R Test Combs detects the Cry1Ac and Cry2Ab proteins produced by a gene, derived from *Bacillus thuringiensis* (*Bt*) and the CP4 EPSPS protein, derived from protein produced by a gene derived from *Agrobacterium* sp. strain CP4. These genes are found in commercial cotton varieties sold under the Bollgard® II and other trade names. The lateral flow test comb has been optimized to easily detect the Cry1Ac, Cry2Ab and CP4 EPSPS proteins expressed in the cotton seed and leaf. The lateral flow strips in this package are sufficient to detect the presence or absence of the Cry1Ac, Cry2Ab and CP4 EPSPS proteins in up to 48 cotton leaf or cottonseed samples in both field and laboratory environments.

### Principle of the Assay

The assay uses a double antibody sandwich format. Antibodies specific to the Cry1Ac, Cry2Ab and CP4 EPSPS proteins 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 Cry1Ac, Cry2Ab or CP4 EPSPS 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 four capture zones, three capture the bound Cry1Ac, Cry2Ab and/or CP4 EPSPS proteins 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

	<u>Quantity</u>
Seed✓ B2R Test Strips	50 or
Seed✓ B2R Test combs	6 combs of 8 strips (48 Strips total)

### Materials Required but not Supplied for

#### Cottonseed Testing:

Trait✓ Sample Buffer Concentrate (7000006)  
 Cell culture plates: 48-well  
 Seed crusher: to fit 48-well cell culture plate  
 Rubber mallet  
 Repeater pipette  
 Brush: stiff bristle  
 Microtiter plate mixer (optional)  
 User Guide (Supplied on request)

### Preparation and Storage 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✓ B2R Test Combs and Trait✓ Sample Buffer can be stored at room temperature or refrigerated. **Do not freeze.** Once opened the Seed✓ B2R lateral flow test strips must be stored in the closed foil bag with the indicating desiccant pack. The moisture-indicating desiccant pack must be blue in color. If the moisture-indicating desiccant pack is pink, contact SDI Technical Service. Storage conditions higher than room temperature may adversely affect performance.

## **Purpose**

The Seed✓ B2R Test Comb has been designed to screen cottonseed and cotton leaf samples for the presence of the Cry1Ac, Cry2Ab and/or CP4 EPSPS proteins. 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.

### **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 0.75ml 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✓ B2R 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**

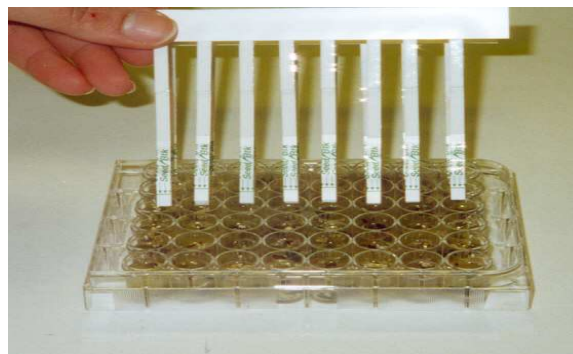
1. Fold a fresh cotton leaf twice and place the four thickness' between the body and cap of a 1.5 ml sample tube and snap the cap into place.
2. Open the cap and remove the excess leaf tissue from around the tube opening.
3. Push the leaf tissue discs into the tube with the stirrer. (This provides about 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✓ B2R 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✓ B2R 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.

### **Multiple Cottonseed Sample Preparation**

#### **Procedure:**

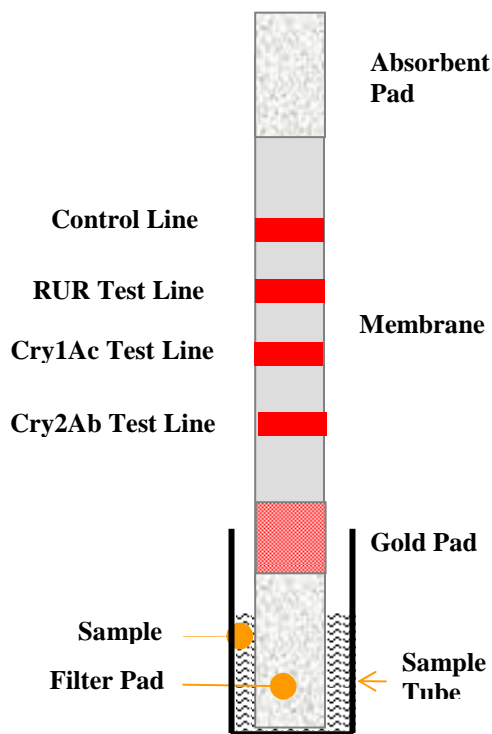
1. Place one cottonseed in each well of the 48-well cell culture plate. Use multiple plates for samples larger than 48 seeds.
2. Place the protective base under the cell culture plate. Be sure it fits properly into the base of the plate.
3. Place the seed crusher "fingers" into the cell culture plate wells and place the combined units on a sturdy base or table.
4. Using a rubber mallet, firmly hit the seed crusher half a dozen or more times at different parts of the seed crusher to uniformly crush the cottonseeds. Use caution to hit evenly to avoid cracking the cell culture plate.
5. Remove the seed crusher from the cell culture plate. Be sure that all seeds are crushed.
6. With the repeating pipette add 0.75 mL of buffer to each well.
7. Place the cell culture plate(s) on a microtiter plate mixer and set the timer for five minutes. The mixer speed should provide good mixing without spilling any buffer from the wells. Alternatively, the plate can be agitated by hand for 5-10 seconds each minute for the five-minute period.
8. Place a lateral flow comb in each row and let sit for 5 minutes. Read the test results.



### Cleaning the seed crusher:

Thoroughly brush the seed crusher “fingers” to remove any cottonseed residue. This will usually be sufficient to avoid carryover. Optionally, after brushing, the seed crusher “fingers” can be sprayed with methanol using a spray bottle. The seed crusher should sit for a few minutes to dry if sprayed with methanol.

### Illustration of a Lateral Flow Strip



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

### Interpreting the Lateral Flow Strip Test

Check the test strips in 5 minutes after inserting the 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 RUR Test Line and indicates a positive result for CP4 EPSPS protein. A red line appearing below the RUR Test Line is the Cry1Ac Test Line and indicates a positive result for Cry1Ac protein. A red line appearing below the Cry1Ac Test Line is the Cry2Ab Test Line and indicates a positive result for Cry2Ab protein. If the test strip displays four (4) red lines, the test is complete and the sample is positive for RUR/Cry1Ac/Cry2Ab cotton traits. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for all three transgenic traits. If no control line develops, the result is inconclusive and need to be repeated.

### Illustration of Positive and Negative Results



Example of test strip results (left to right)

- 1) unreacted
- 2) negative (non-GMO)
- 3) RUR positive
- 4) Cry1Ac positive
- 5) RUR and Cry1Ac positive
- 6) RUR, Cry1Ac and Cry2Ab positive

## 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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