Strategic Diagnostics Inc. *Test and Be Sure*

Trait✓NK603 RUR Test Kit

Part Number 7000011

Application for Bulk Canola Testing

Intended Use

The intended use of the kit is the qualitative (yes/no) determination of the CP4 EPSPS protein in canola bulk seed and grain samples. The Trait✓ NK603 RUR test strip has a detection limit of one (1) Roundup Ready® (CP4 EPSPS) canola seed in 1000 non-RUR canola seeds.

Product Description

The Trait✓ NK603 RUR Test Strip detects the CP4 EPSPS protein expressed by a gene derived from *Agrobacterium* sp. strain CP4. These genes have been incorporated into herbicide-resistant soybean, corn, cotton, canola and other crops including Roundup Ready® brands from Monsanto and other companies. The lateral flow test strip has been optimized to easily detect the CP4 EPSPS protein expressed in the bulk grain, seed and leaf. The lateral flow strips in this package are sufficient to detect the presence or absence of the CP4 EPSPS protein in up to 100 bulk grain samples. This product can screen Roundup Ready® canola grain at specified levels.

Principle of the Test

The assay uses a double antibody sandwich format. Antibodies specific to the CP4 EPSPS 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 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 two capture zones, one captures the bound CP4 EPSPS 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✓ NK603 RUR 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 oz.) (6000033)
Blender blades, pulverizer (6000040)
Sample tube rack (6000023)
Graduated cylinder, 50 ml (6000036)

Detection Limits

Screening grain at very low GM levels can be accomplished by using a sufficiently large sample size that test 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✓ RUR NK603 strip test sensitivity is at least one RUR canola seed in 1000 seeds.**

Note: Samples less than 500 seeds should be ground by hand (mortar and pestle) to assure all seeds are processed

Principle of the Screening Application

This test protocol does not determine the exact percent of GMO canola seeds. It determines the probability that a sample contains greater or less than a specified threshold concentration. For RUR canola, if one 1000-seed sample is negative by the Trait✓ test strips, there is a 95% confidence that the sample contains less than 0.30% GMO content. Refer to the table below for other confidence levels with multiple 1000-seed sub-samples.

1000 Seed Sub-Samples
(All Sub-Samples Must be Negative)

No. Sub-Samples of 1000 Seeds	Percent GM using Sub-Sample Sizes of 1000 Seeds at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	0.07	0.14	0.23	0.30	0.46
2	0.035	0.07	0.12	0.15	0.23
3	0.024	0.047	0.08	0.10	0.16

Preparation and Storage of Reagents

The Trait✓ Test Kits should be stored at room temperature. The Trait✓ 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✓ Test Kits 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✓ tests will depend on the percent GMO screening level desired and an acceptable level of risk that the GMO level is close to the screening level.

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: Weigh the Sample

The statistical sampling plan is dependent on the number of canola seeds used. However, it is more practical for routine testing to weigh canola seeds instead of counting to obtain the desired number of seeds. The average weight of canola seeds depends on the variety of canola and environmental conditions.

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

1. Count 100 seeds of the variety to be tested.
2. Weigh the 100 seeds to the nearest 0.01 gram.
3. Divide the weight of the canola seeds by 100 to get the average grams per seed.
4. Multiply this average weight by the desired number of canola seeds in the sub-samples to determine the weight for the sub-samples.
5. Construct a weight-to-canola seed ratio table for each variety for the different sub-sample sizes to be used.

Example: One hundred (100) canola seeds of Variety A weigh 0.35 grams. Each canola seed then weighs 0.0035 grams. Multiply the 0.0035-gram per canola seed times the number of seeds in each sample size to get the following table.

Table A: Example: Weight-to-Seed Ratio

No. Canola Seed (a)	500	750	1,000
Sample Weight (g)	**1.75**	**2.63**	**3.5**

This average weight is then used to obtain the number of seeds for this canola variety

Sample Preparation

The canola 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 canola sample. The volume of water in milliliters (mL) should be 3 times the weight of canola sample in grams (g).

Sample Weight (g) X 3= Water Volume (mL)

The size of "Mason" jar required and the grinding time depends on the sample size to be analyzed. **For 1000-seed samples, a 4-oz. Mason jar is used.**

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** 4-oz. "Mason" jar.
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 10-15 seconds.

Caution: It is recommended to shield the jars during grinding with a "tri-cornered" 1-liter plastic beaker (P/N 6000037).

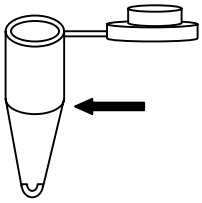
5. Remove the adapter and cutting blades.
6. Add the prescribed volume of water (see above) to the ground canola in the jar, place a lid on the jar and shake the jar until all the ground canola is well wetted (about 10-20 sec.).

Note: The sample will have a "thick" consistency but should contain some free liquid after a short settling time. **There should be no whole seeds 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 a Trait✓ NK603 RUR Test Strip into the sample tube.
3. Let sit for 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.

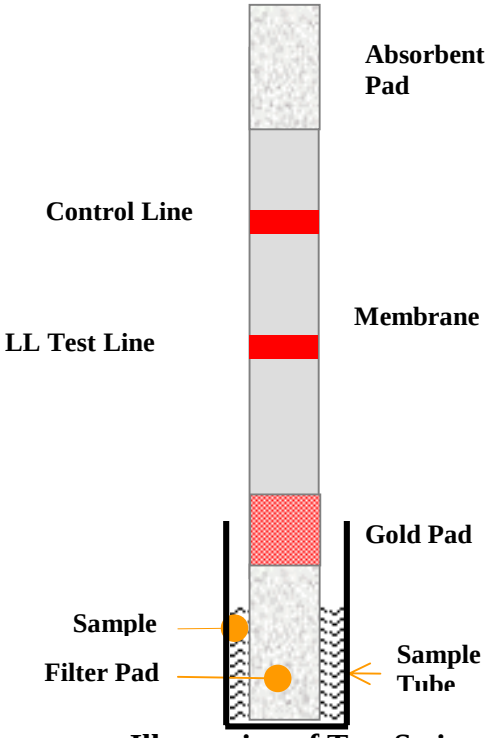


Illustration of Test Strip

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 RUR canola. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for RUR canola. 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.***

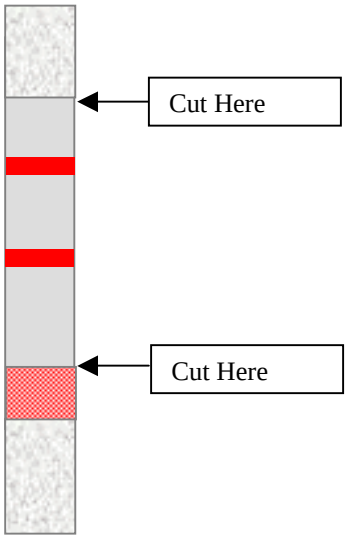
Illustration of Positive and Negative Results

Example of an unreacted, negative (1-line) and positive (2-lines) test strip

***Note*: Refer to the specific Trait✓ Test Kit user's guides for detailed summary of the test procedures, and performance.**

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 blender jar and blades between samples.

1. The blender jar should be emptied, rinsed thoroughly with water and completely dried with a paper towel between uses.
2. The blades should be rinsed with water until **all ground canola** 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.

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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