Trait✓ RUR Lateral Flow Test User Guide

Trait✓ RUR NK603 Corn Grain Test Kit (100-Test)
Part Number 7000011

Product Description

The Trait✓ RUR Lateral Flow Test Kits are designed to detect the CP4 EPSPS protein produced by a gene derived from *Agrobacterium* sp. strain CP4. This gene has been incorporated into Roundup Ready® crops including soybeans, canola, cotton, corn and others. The intended uses of the kits include the qualitative (yes/no) determination of the CP4 EPSPS protein in plant tissue and crop samples. The lateral flow strips and other components provided in the kits are sufficient to make qualitative determinations for the presence or absence of the CP4 EPSPS protein in both field and laboratory environments. Different application protocols are required for different crops, seeds and grains and for the determination of different expected amounts of the CP4 EPSPS protein in the samples. **This application is intended for the detection of NK603 Roundup Ready® corn only (RUR NK603). It does not detect genetic event GA21 Roundup Ready® corn.**

Principle of the Assay

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, an antibody sandwich is formed with some, but not all the antibody that is coupled to the color reagent. This colored sandwich then flows through the porous membrane. The membrane contains two capture zones, one specific for the CP4 EPSPS protein sandwich and one specific for unreacted antibodies coupled to the 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✓ RUR Test Strips	100
Trait✓ Sample Buffer, Grain (30 ml)	1
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 and 8 oz.)
Sample tube rack (6000023)
Graduated cylinder, 250 ml (6000024)

Storage and Preparation of Reagents

The Trait✓RUR NK603 Lateral Flow Test Kit should be stored at room temperature. The Trait✓RUR Lateral Flow Strips must be kept in the foil pouch with desiccant. Storage conditions higher than room temperature may adversely affect performance.

Sampling

The samples used for the Trait✓ RUR NK603 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✓ RUR test will depend on the level of RUR NK603 corn at which the screening is being conducted and an acceptable level of risk that the GM level is close

to the screening level. The number and size of the sub-samples will be discussed in more detail in the application protocol 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)	50	100	150	200
Sample Weight (g)	**12.5**	**25.0**	**37.5**	**50.0**

(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
125-200	8	15-30

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**.

Note: It is important to clean and dry the jars and cutting blades between samples.

Equipment Cleaning and Drying

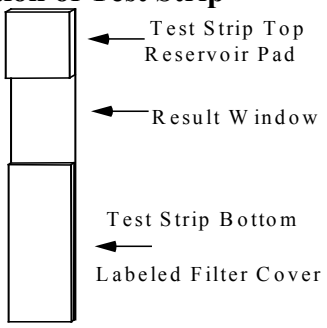
1. The Mason jar should be emptied, rinsed well with water and completely dried with a paper towel between uses.
2. The cutting blades for the blender should be wiped clean, sprayed or rinsed with methanol and dried with a paper towel between uses.

Test Procedure

1. Place 0.5 mL of the sample extract prepared above to a 1.5-mL sample tube using the transfer pipette provided.
2. Add three (3) drops of the Trait✓ Sample Buffer into the sample tube.
3. Mix well.
4. Place one Trait✓ RUR Test Strip into the sample tube. Let sit for 5-10 minutes
5. The appearance of one line (control) on the strip indicates a negative result.
6. The appearance of two lines on the strip indicates a positive result.

Interpreting the Lateral Flow Strip Test

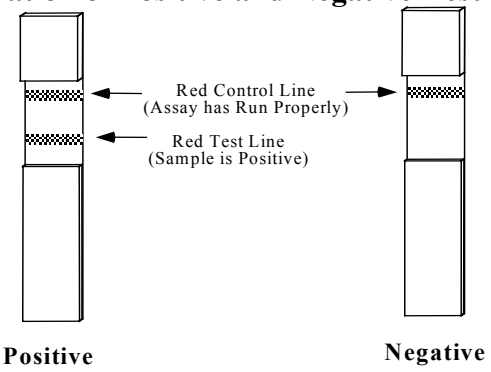
Illustration of Test Strip



Check the result window at about 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 NK603 corn. If at about 10 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for RUR NK603 corn.

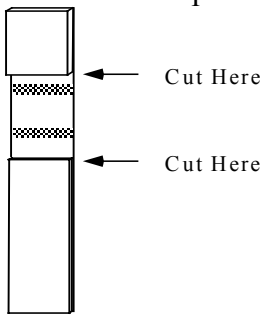
Note: ***Test strip results should be interpreted after about 10 minutes. Test strips interpreted after 60 minutes are invalid.***

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.



Principle of the Screening Application

The Trait✓ RUR Test Strip provides a yes/no answer for the presence or absence of RUR NK603 corn in a given sample. Testing multiple statistically selected sub-samples allows an estimate of the percent of RUR NK603 corn. The test results provide information about the probability of the percent RUR NK603 corn in the sample.

Note: The test protocol does not determine the exact percent of RUR NK603 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 RUR NK603 corn). This Distribution can determine the probability of having no RUR NK603 kernels in a random sample of a given number of kernels at a given percent RUR NK603. For example, a random sub-sample of 100 corn kernels selected from a larger population containing one- percent RUR NK603 corn has a 36.8% probability of containing no RUR NK603 corn kernels. The probability of a 75-corn kernel sub-sample (at one percent RUR NK603) containing zero RUR NK603 corn kernels is 47.2%.

Screening at Very Low RUR NK603 Levels

Screening grain at very low RUR NK603 levels can be accomplished by using a sufficiently large sample size that tests negative for the RUR NK603 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 kernel in 200.**

The following tables provide information at five confidence levels with the use of multiple samples of 70 kernels 125 kernels or 200 kernels each. The tables provide the maximum percent RUR NK603 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: 70 Seed Sub-Samples (All Sub-Samples Must be Negative)

No. Sub-Samples of 70 Seeds Each	Percent RUR NK603 using Sub-Sample Sizes of 70 Seeds at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	1.0	2.0	3.3	4.3	6.6
2	0.50	0.99	1.65	2.15	3.3
3	0.33	0.66	1.43	1.65	2.20
4	0.25	0.50	0.82	1.07	1.65
5	0.20	0.40	0.66	0.86	1.32
6	0.17	0.33	0.55	0.71	1.10
7	0.14	0.28	0.47	0.61	0.94
8	0.12	0.25	0.41	0.53	0.82

Table D: 125 Seed Sub-Samples (All Sub-Samples Must be Negative)

No. Sub-Samples of 125 Seeds Each	Percent RUR NK603 using Sub-Sample Sizes of 125 Seeds at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	0.56	1.1	1.9	2.4	3.7
2	0.28	0.56	1.0	1.2	1.9
3	0.19	0.37	0.65	0.80	1.3
4	0.14	0.28	0.46	0.60	0.93
5	0.11	0.23	0.37	0.48	0.75
6	0.093	0.19	0.31	0.40	0.65
7	0.080	0.16	0.27	0.34	0.55
8	0.070	0.14	0.23	0.30	0.48

Table E: 200 Seed Sub-Samples (All Sub-Samples Must be Negative)

No. Sub-Samples of 200 Seeds Each	Percent RUR NK603 using Sub-Sample Sizes of 200 Seeds at Five Different Confidence Levels (%)				
	50	**75**	**90**	**95**	**99**
1	0.35	0.70	1.1	1.5	2.5
2	0.17	0.35	0.58	0.75	1.2
3	0.12	0.23	0.39	0.50	0.78
4	0.087	0.18	0.29	0.38	0.58
5	0.070	0.14	0.24	0.30	0.47
6	0.058	0.12	0.20	0.25	0.39
7	0.050	0.10	0.18	0.22	0.35
8	0.045	0.085	0.15	0.19	0.29

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 RUR NK603 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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For Technical Service call:

Strategic Diagnostics Inc
111 Pencader Drive
Newark, DE 19702

Phone: 800-544-8881
Fax: 302-456-6782
e-mail: techservice@sdix.com