



Trait✓ RUR Lateral Flow Test User Guide

Trait✓ RUR Bulk Soybean 5-Minute Test Kit (100-Test)
Part Number 7000014

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 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.

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

<u>Description</u>	<u>Quantity</u>
Trait✓ RUR Test Strips	2 x 50
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, 8, 16 and 32 oz.)
- Sample tube rack (6000023)
- Graduated cylinder, 250 ml (6000024)

Storage and Preparation of Reagents

The Trait✓ RUR Lateral Flow Test Kit should be stored at room temperature. The Trait✓ RUR Test 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✓ RUR Bulk Soybean 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 GM soybeans 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 soybeans used. It is more practical to weigh soybeans to obtain the correct sub-samples size, however, the number of soybeans in a unit weight depends on the variety of soybean and environmental conditions.

Table A is a guideline for the weight to soybean number ratio.

Table A: Average Weight of Seeds (Assoc. of Official Seed Analysts)

<u>Seed Size</u>	<u>Ave. Grams / Seed</u>
Very Petite	0.076
Small	0.095
Medium	0.223
Large	0.293

However, it is recommended that the ratio for each variety be determined as follows.

1. Count 100 soybeans of the variety to be tested.
2. Weigh the 100 beans to the nearest 0.01 gram.
3. Divide the weight of the soybeans by 100 to get the average grams per soybean (the result will be less than one gram per soybean).
4. Multiply this average weight by the desired number of beans in the sub-samples to determine the weight for the sub-samples.
5. Construct a table for each variety for the different sub-samples sizes to be used.

Example: One hundred (100) soybeans of Variety

X weigh 15.00 grams. Each soybean then weighs 0.15 grams. Multiply the 0.15 gram per soybean times the number of soybeans in each sample size to get the following table.

Example of Weight-to-Soybean Ratio Table

	Variety X: Grams per Sample			
Soybeans (#)	60	125	250	700
Weight (g)	9.00	18.75	37.50	105.0

This average weight is then use to determine the weight of soybeans that will be used to represent the number of soybeans (for this variety) selected in the Confidence Tables.

Sample Preparation: Processing the Sample

The following table contains recommendations for the size of jar, approximate liquid volume and grinding time for sample extraction. The seed sample size is at the 50% confidence levels (see **Principle of Screening Application** section). The suggested processing parameters were determined using the laboratory grade Waring Model 31BL91 food processor and mid-sized soybean varieties. Other food processors or extreme sized soybeans will require further validation.

Table B: Parameters for Preparing Samples

GM Screen (%)	Number of Seeds	Jar Size (oz.)	Water Volume (ml)	Grind Time (sec)
2	60	4	70	10
1	125	4	70	10
0.5	250	8	140	20
0.1	700	16	350	30
<0.1	1000	32	700	30

1. Weigh sub-samples from each truck or container.
2. Place each sub-sample in a **dry** “Mason” jar.
3. Attach the jar adapter and **dry** cutting blades.
4. Place the jar onto the food processor and grind the sub-samples for the specified time.
5. Remove the adapter and cutting blades.
6. Add water to the ground soybeans in the jar (to the bottom of the neck or the approximate

volumes in Table B), place a lid on the jar and shake the jar until all the ground soybeans are well wetted (about 10-20 sec.)

7. Use this sample extract for 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.

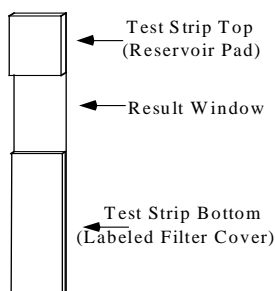
Test Procedure

1. Place 0.5 ml of the sample extract prepared above to a 1.5-ml sample tube using the transfer pipette provided.
2. Place one Trait✓RUR Test Strip into the sample tube. Let sit for 5 minutes. The appearance of one line (control) on the strip indicates a negative result.
3. The appearance of two lines on the strip indicates a positive result.

Reading the Lateral Flow Test Strip

1. Insert the labeled Filter Cover of the Trait✓RUR test strip into the sample tube containing the buffered soybean sample extract. The arrows on the filter cover should point into the tube.

Illustration of Test Strip

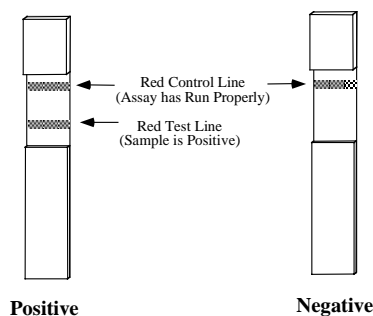


2. Check the result window frequently after adding the strip. At least one line, the Control Line, should always develop approximately one (1) cm down from the Reservoir Pad. A

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 Roundup Ready® soybeans. If at about five (5) minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Roundup Ready® soybeans.

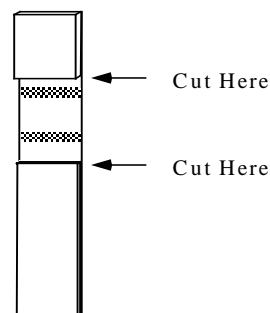
Note: Test strip results should be interpreted after 5 minutes. Test strips interpreted after 60 minutes are invalid.

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 Lateral Flow Test Strip provides a yes/no answer for the presence or absence of Roundup Ready® soybeans in a given sample. One red line at the top of the test strip indicates a negative result and two red lines, the second line lower on the test strip, indicates a positive result for the presence of Roundup Ready® soybeans.

Testing two or three statistically selected sub-samples allows an estimate of the percent of Roundup Ready® soybeans. The test results can provide information about the probability of the percent in the representative sample being within certain ranges.

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

The statistical model used for this application is the Poisson Probability Distribution which provides good approximations to binomial (yes/no) probabilities when the number of items tested (i.e. soybeans) is large but the probability of a positive result is expected to be small (i.e. low level of GM soybeans). Using the Poisson Probability Distribution it is possible to determine the probability of having zero Roundup Ready® soybeans in a random sample of a given size (number of soybeans) at a given percent Roundup Ready® soybeans. For example, a random sub-sample of 100 soybeans selected from a larger sample at one- percent GM soybeans has a 36.8% probability of containing no Roundup Ready® soybeans. The probability of a smaller 75-soybean sub-sample (at one- percent GM) containing no Roundup Ready® soybeans is 47.2%.

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✓RUR strip test sensitivity is at least one RUR bean in 1000 non-GMO beans.**

The following tables provide information at five confidence levels with the use of multiple samples of 125 beans 400 beans or 1000 beans 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. For other sample sizes or testing criteria, please contact SDI Technical Service.

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

No. Sub-Samples of 125 Seeds Each	Percent GM 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 F: 400 Seed Sub-Samples
(All Sub-Samples Must be Negative)**

No. Sub-Samples of 400 Seeds Each	Percent GM using Sub-Sample Sizes of 400 Seeds at Five Different Confidence Levels (%)				
	50	75	90	95	99
1	0.17	0.35	0.58	0.75	1.2
2	0.087	0.18	0.29	0.38	0.58
3	0.058	0.12	0.20	0.25	0.39
4	0.045	0.085	0.15	0.19	0.29
5	0.035	0.070	0.12	0.15	0.25
6	0.029	0.058	0.10	0.13	0.20
7	0.025	0.050	0.085	0.11	0.18
8	0.022	0.044	0.075	0.10	0.15

**Table G: 1000 Seed Sub-Samples
(All Sub-Samples Must be Negative)**

No. Sub-Samples of 1000 Seeds Each	Percent GM using Sub-Sample Sizes of 1000 Seeds at Five Different Confidence Levels (%)				
	<u>50</u>	<u>75</u>	<u>90</u>	<u>95</u>	<u>99</u>
1	0.070	0.139	0.231	0.300	0.461
2	0.035	0.070	0.116	0.150	0.231
3	0.024	0.047	0.077	0.100	0.155
4	0.018	0.035	0.058	0.075	0.116
5	0.014	0.028	0.047	0.060	0.093
6	0.012	0.024	0.039	0.050	0.077
7	0.010	0.020	0.033	0.043	0.066
8	0.009	0.018	0.029	0.038	0.058

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