



Strategic Diagnostics Inc.



Test and Be Sure

Trait✓ *RUR HS Test Kit*

Part # 7000011

Application for Bulk Alfalfa Seed Testing



Intended Use

The intended use of this application is the qualitative (yes/no) determination of the CP4 EPSPS protein in alfalfa bulk seed samples. The Trait✓ RUR HS test strip has been validated for a detection limit of one (1) Roundup Ready® alfalfa seed in 600 conventional alfalfa seeds in 5 minutes.

Application Description

The Trait✓ RUR HS Test Strip detects the CP4 EPSPS protein expressed by a gene derived from *Agrobacterium* sp. strain CP4. This gene has been incorporated into herbicide-resistant crops in the Roundup Ready® brands (RUR) from Monsanto and other companies. The lateral flow test strip has been optimized to detect the CP4 EPSPS protein expressed in the bulk seed samples. 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 seed samples. The Trait✓ RUR HS test strip can provide a qualitative, threshold determination of the CP4 EPSPS protein in alfalfa samples consisting of up to 600 seeds.

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

<u>Description</u>	<u>Quantity</u>
Trait✓ RUR HS Lateral Flow Test Strips	2x50*
OR Trait✓ RUR NK603 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

Sample cups, 4 oz. (1034053)
Coffee grinder
Tap water

Detection Limits

Determining the presence of low levels of the CP4 EPSPS protein in alfalfa seeds can be accomplished by using a sufficiently large sample size. Lateral flow strips can be used by testing multiple 600 seed sub-samples. **The Trait✓ RUR HS strip test sensitivity is at least one Roundup Ready alfalfa seed in 600 seeds.**

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 closed canister with the desiccant. The moisture indicator card must be blue in color. Avoid prolonged exposure of the strips to moisture and humidity by **tightly closing** the canister once the required number of strips are removed. Storage conditions higher than room temperature (15-30°C) may adversely affect performance. Refrigerate the test strips if room temperature storage will be greater than 30°C for more than 1 week.

Sampling

The samples used for the Trait✓ Test Kits can be sub-samples of those “representative samples” collected from seed containers for other tests. The size of the sub-sample to be used for the Trait✓ RUR Alfalfa test has been optimized for 600 alfalfa seeds.

Note: It is assumed that the samples collected are representative of the contents of the 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 alfalfa seeds used. However, it is more practical for routine testing to weigh alfalfa seeds instead of counting to obtain the desired number of seeds. The average weight of alfalfa seeds depends on the variety of alfalfa and environmental conditions.

It is recommended that the weight-to-alfalfa 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 alfalfa seeds by 100 to get the average grams per seed.
4. Multiply this average weight by 600 alfalfa seeds to determine the weight for the sub-samples.

Sample Preparation

The alfalfa sample is ground in a coffee grinder and then transferred to the sample container for extraction with water. The volume of water in milliliters (mL) should be 10 times the weight of alfalfa sample in grams (g).

Sample Weight (g) X 10= Water Volume (mL)

The processing parameters were determined using a standard coffee grinder (see **Materials Required but not Supplied**).

- 1) Transfer the 600 seed sample to a clean coffee grinder
- 2) Place the lid onto the coffee grinder and pulse grind the sample using six pulses of 5 second each. Allow the blades to come to a stop between pulses. **There should be no whole seeds remaining – re-grind if whole seeds remain.**
- 3) Transfer the entire sample back to its sample container. Be sure to carefully clean the grinder between samples, removing all traces of the previous sample.
- 4) Add the prescribed volume of tap water to the ground alfalfa in the jar, place a lid on the container and shake the container for 10 to 20 seconds, being sure the entire sample is well wetted.

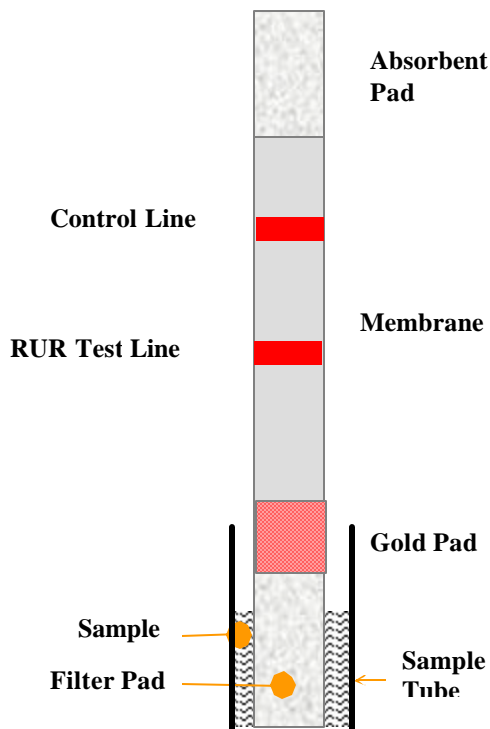
Test Procedure

1. Remove the lid from the sample container. Place the Trait✓ RUR HS strip directly into the liquid. **The arrows must face down into the sample.** It does not matter whether the bottom of the strip sits on or under the sediment in the bottom of the cup. The upper portion of the strip may rest against the side of the container. The sample liquid level should be below the filter pad (~0.5 cm). Alternatively, the sample extract can be transferred to a Sample Tube with the transfer pipette provided with the test kit (~0.5 mL).



2. Let sit for 5 minutes.
3. The appearance of **one line** (control) on the strip indicates a **negative** result.
4. The appearance of **two lines** on the strip indicates a **positive** result for Roundup Ready.

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 Roundup Ready alfalfa. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is negative for Roundup Ready alfalfa. If no control line develops, the result is inconclusive and needs to be repeated.

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

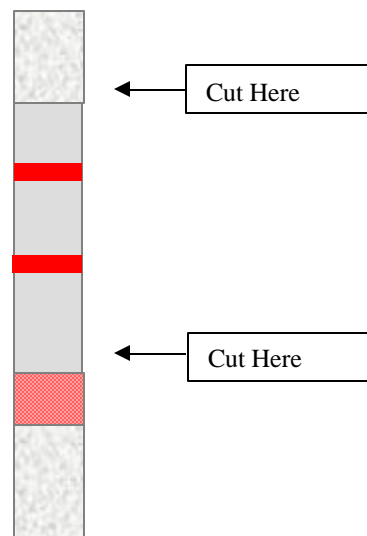
Illustration of Positive and Negative Results



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

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

This test protocol is not designed to measure the exact percent of alfalfa seeds containing CP4 EPSPS protein in a given sample. This test protocol determines the probability that a sample contains greater or less than a specified threshold concentration. For an alfalfa sample, if one 600-seed sample is negative by the Trait✓ test strips, there is a 95% confidence that the sample contains less than 0.50% seeds expressing CP4 EPSPS protein or Roundup Ready alfalfa seeds. Refer to the table below for other confidence levels with multiple 600-seed sub-samples.

Statistical Interpretation

The following tables provide information at three confidence levels with the use of multiple samples of 600 seeds each. The tables provide the maximum percent RUR levels that would be expected in the sample if 0, 1 or 2 test-samples provide **positive** results when 1-3 sub-samples are tested.

Table 1: No Sub-Samples are Positive

No. Sub-Samples of 600 Seeds Tested	No. of POSITIVE Sub-samples	Percent RUR using Sub-Sample Sizes of 600 Seeds at Three Different Confidence Levels (%)		
		<u>90</u>	<u>95</u>	<u>99</u>
1	0	0.38	0.50	0.77
2	0	0.19	0.25	0.38
3	0	0.13	0.17	0.26

Table 2: 1 Sub-Sample is Positive

No. Sub-Samples of 600 Seeds Tested	No. of POSITIVE Sub-samples	Percent RUR using Sub-Sample Sizes of 600 Seeds at Three Different Confidence Levels (%)		
		<u>90</u>	<u>95</u>	<u>99</u>
2	1	0.49	0.61	0.88
3	1	0.27	0.33	0.47

Table 3: 2 Sub-Samples are Positive

No. Sub-Samples of 600 Seeds Tested	No. of POSITIVE Sub-samples	Percent RUR using Sub-Sample Sizes of 600 Seeds at Three Different Confidence Levels (%)		
		<u>90</u>	<u>95</u>	<u>99</u>
3	2	0.56	0.68	0.95

Equipment Cleaning and Drying

Caution: It is important to clean and dry the grinder between samples.

The grinder should be emptied, wiped down with moist towels thoroughly and completely dried with a paper towel between uses. Special attentions should be paid to the area at the base of the blades. It is not advisable to put water directly into the grinder. **BE SURE TO UNPLUG THE GRINDER WHEN CLEANING.**

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