



# RAPID TEST

## CP4 EPSPS

### Lateral Flow Test Kit

### for the determination of CP4 EPSPS protein in corn, soybean & canola seeds

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A.

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

**Use only the current version of Product Data Sheet enclosed with the kit.**

Rapid Test CP4 EPSPS, R2130/R2160, is a lateral flow test for the determination of the presence of CP4 EPSPS protein at the levels typically expressed in Roundup Ready crops. This kit contains all reagents required for 30 or 60 reactions.

#### Matrices:

Corn ,soybean & canola seeds.

- Sample preparation: extraction
- Test time: 5min
- Range: 0 - 2% GMO
- Shelf life: 12 months
- Storage: 2-8°C

## 1. Description

Rapid Test CP4 EPSPS is a Lateral Flow test for the quantitative detection of CP4 EPSPS protein at the levels typically expressed in Roundup Ready crops, more specifically in corn, soybean and canola seeds.

## 2. General information

Genetically Modified Organisms (GMOs) are organisms whose genetic material has been altered through genetic engineering techniques. They are commonly used in agriculture to confer traits such as resistance to pests, diseases and herbicides. One essential component in many genetically modified crops is the CP4 EPSPS protein, derived from the bacterium *Agrobacterium* strain CP4. The expression of the CP4 EPSPS protein provides plants with resistance to glyphosate-based herbicides like Roundup.

## 3. Principle of the method

The Rapid Test CP4 EPSPS is a sandwich based immunochromatographic lateral flow assay. The detection system consists of specific antibodies against CP4 EPSPS conjugated with colloidal gold. During testing, the sample is carried through the membrane by capillary action, bringing along the detection system and passing through the test (T) and control (C) lines. If CP4 EPSPS protein is present in the sample, it binds with the gold-labeled antibodies, forming a complex that is captured by the test line, resulting in the development of a red color. Conversely, if CP4 EPSPS protein is absent, the test line will not appear. A valid test will always display a red upper control line. The test line color intensity can be utilized to quantify the amount of CP4 EPSPS protein present in the sample.

## 4. Reagents provided

The Rapid Test CP4 EPSPS kit contains sufficient reagents and materials for 30/60 measurements.

- 30/60 tests (cassette format) in foils
- Instruction manual

## 5. Materials required but not provided

- A grinder sufficient to render sample homogenized
- Balance with 0 - 50g measuring capability
- Graduated cylinder 5-50ml
- Clean and dry container
- Tap water
- Timer
- 100µL adjustable single channel micropipette with disposable tips
- S-Flow software along with matching scanner device provided by lateral logic ltd

## 6. Storage instructions

Store kit components between 2 - 8°C. Do not freeze any components provided. The expiry date of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated due to prior handling. Do not interchange individual components between kits of different lot numbers.

## 7. Safety and precautions for use

All reagents should be brought to room temperature (21 - 25°C) before use (at least half an hour). Do not re-use any of the kit components to avoid cross contamination.

## 8. Sample preparation

### 8.1 Determine number and size of sub-samples

1. The sample must be collected according to established sampling techniques.<sup>1</sup>
2. To select the appropriate sample size, determine the purity standard and the degree of confidence required. Confidence level means the statistical probability that the true Roundup Ready level in the lot is below the selected purity standard. Table 1 provides a guideline for determining the number of beans in each sub-sample that are necessary to provide effective screening for different GM concentrations at the 95% and 99% confidence levels.

**Table 1. Soybeans - Number of beans per sub-sample at different GM concentrations**

Testing 2 sub-samples one or both sub-samples being negative (-/-) or (-/+)							
Confidence level (%)	Roundup Ready screening level Soybeans						
	5%	4%	3%	2%	1%	0.5%	0.4%
95%	72	90	120	182	366	734	915
99%	104	130	175	262	527	1060	—
Testing 3 sub-samples two or all three sub-samples being negative (-/-) or (-/-/+)							
95%	39	49	66	100	200	400	1000
99%	55	70	93	141	282	565	—

Note: Screening at the 0.1% Roundup Ready concentration level, with 95% confidence, would require testing 3 sub-samples of 1000 beans with all 3 sub-samples negative (-/-/-).

### 8.2 Determine sub-sample weight, jar size and grid times

1. Determine average weight of individual grain to be tested (weigh 100 beans, divide by 100).
2. Calculate the weight of the number of grains to be tested (Number of grains X Average Weight/Grain).
3. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh sieve). For soybeans seeds, grid according to table 2.

**Table 2. Grinding time and sample weight**

Commodity	Sample weight (g)	Jar size (oz)	Grid time (Sec)
Soybeans	15 - 59	16	45
	60 - 100	32	60

### 8.3 Sample extraction

1. Weigh out a 2g ground portion of the sample in a clean container/tube.
2. Add the appropriate volume of tap water according to table 3.

**Table 3. Sample weight and water ratio**

	Sample weight (g)	Volume of water (mL)	Ratio Sample (g): water (mL) (w/v)
Soybeans	2	40	1:20
Corn	2	10	1:5
Canola	2	10	1:5

3. Cap and shake for a minimum of 1 minute. Make sure the entire sample is wet.

4. Allow sample for a few minutes to settle. The sample extract (liquid from the top) is ready to be analyzed.

Note: In case the sample is not analyzed within 10 minutes after extraction, transfer a sufficient amount of the extract in a clean tube and analyze later. Do not freeze the extract. Do not use thawed extract.

## 9. Method procedure

1. Before opening the reagents, take the kit out of the fridge (at least for half an hour) and wait until the temperature of the reagents reaches the ambient temperature.
2. Open as many foils with cassettes as the number of samples to be tested.
3. Place a new tip on the single channel micropipette and transfer **100µL** of the sample extract in the circular window of the cassette.
4. Set the timer for 5 minutes.
5. When the 5 minutes are over, place the cassette inside the plastic holder and press SCAN using the S-Flow software. The cassette must be facing up. The software will use a Lot specific curve to calculate the result as % GMO.

Note: The standard curve for the quantification of the CP4 EPSPS protein in the sample was calculated by reference material. Actual field expression may vary.

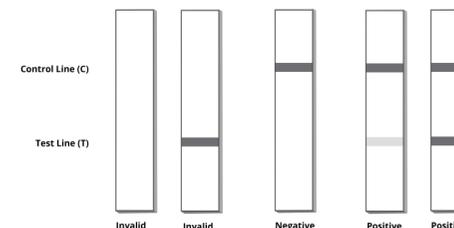
## 10. Specifications

### 11. Visual interpretation

Matrix	Limit of detection (LOD)	Limit of quantification (LOQ)
Soybeans	0.04%	0.05%
Corn	0.10%	0.12%
Canola	0.03%	0.05%

When the test time is completed (5 min), the test can also be visually read and interpreted according to the following figure.

Visual result interpretation index



1. The control line should always be visible, if not the test is invalid.
2. If the test line is absent, the sample is negative.
3. If the test line appears, the sample is positive.

## 12. Performance evaluation

### 12.1 Reference Materials

Several reference materials are being used for the evaluation of each product of Prognosis Biotech S.A. in the context of Quality Control Department. Please request a validation report, including the results, at [exports@prognosis-biotech.com](mailto:exports@prognosis-biotech.com).

### 12.2 Proficiency Tests

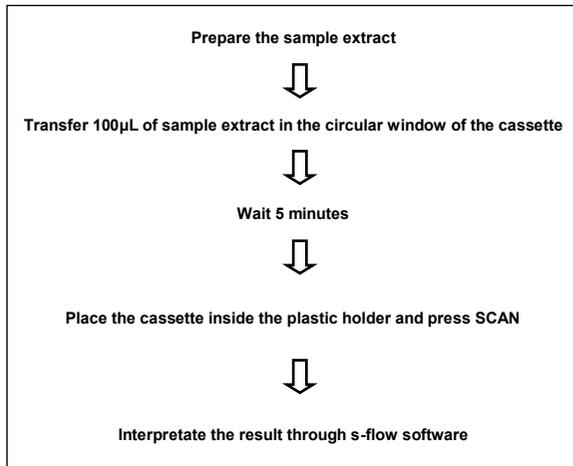
All products participate frequently in Proficiency Tests. For more information, visit the individual product page on our website: [www.prognosis-biotech.com](http://www.prognosis-biotech.com)

## 13. References

1. USDA Grain Inspection Handbook, Book I Sampling.

## 14. Method summary

Total method time: 5 minutes.



All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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R2130-R2160 Manual\_Rapid Test\_CP4\_v2\_en



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