# CP4EPSPS ELISA Kit

## Cat No. AID 011

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## Intended use

CP4EPSPS ELISA kit (Roundup Ready**/CP4) is intended to be used for the qualitative detection of CP4 in individual soybean, cotton or corn leaf/seed samples.

The total incubation time of the assay is 60 minutes.

## Principle of the test

An antibody specific to the CP4 protein molecule is immobilized on the microwell plates and a second antibody specific for the CP4 molecule is conjugated with horseradish peroxidase (HRP). When sample extracts are added to the microtiter wells, the CP4 from the sample binds to the antibody immobilized in the well. This binding is subsequently detected by the addition of an enzyme-labeled antibody. After a washing step, the substrate is added. The enzymatic reaction and development of color is proportional to the amount of CP4 present in the sample. The reaction is terminated by the addition of the stopping solution. Absorbance is then measured on a plate reader.

Light color indicates a lower concentration, while a dark color indicates higher concentration of the CP4 protein in the sample.

## Cross-reactivity

The CP4EPSPS (Roundup Ready**) ELISA Kit does not recognize Cry1F, Cry2A, Cry9C or PAT/pat (Liberty Link***)

## Precautions:

The CP4EPSPS (Roundup Ready**) ELISA Kit is intended for in vitro use only. The reagents contain Thimerosal as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

## Storage of the kit

The kit should be stored at 2 - 8°C. The unopened kit is stable till the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the component.

## Contents of the kit:

**Kits are provided in 10 plates or 50 plates format.**

**Contents of 10-plate kit:**

1. Ten plates of 96 wells coated with Anti-CP4EPSPS antibody, individually packed in a laminated bag with silica gel
2. CP4EPSPS enzyme conjugate: One ready-to-use bottle of 55 ml
3. Extraction buffer concentrate: One packet of powder with a 25ml vial of Tween-20
4. Wash solution concentrate: One packet of powder with a 2.5 ml vial of Tween-20
5. TMB Substrate: One ready-to-use bottle of 110 ml
6. CP4EPSPS Negative control: one vial of 10ml reagent, negative for CP4EPSPS

**Contents of 50-plate kit:**

1. Fifty plates of 96 wells coated with Anti-CP4EPSPS antibody, individually packed in a laminated bag with silica gel
2. CP4EPSPS conjugate: One ready-to-use bottle of 275 ml
3. Extraction buffer concentrate: One packet of powder with a 25ml vial of Tween-20
4. Wash solution concentrate: One packet of powder with a 2.5 ml vial of Tween-20
5. TMB Substrate: One ready-to-use bottle of 500ml
6. CP4EPSPS Negative control: One vial of 10ml reagent, negative for CP4EPSPS

## Material and equipment required but not provided

- Pipette with disposable plastic tips
- Multichannel pipette with disposable pipette tips
- Deionized or distilled water
- Graduated cylinders of one litre capacity
- Reagent troughs
- ELISA microplate washer or wash bottle
- ELISA microplate reader with 450/620 nm filter
- Table top centrifuge
- Marking pen, Parafilm*, pipette tips, timer and paper towels

## Reagent preparation

**The procedure for preparation of the reagent is as follows:**

**Preparation of working extraction buffer:**

Dissolve each packet of extraction buffer concentrate powder in 500 ml of distilled/deionised water and add 25 ml of Tween-20 which is provided along with the powder. Mix it well before use. The resultant extraction solution is 10X concentrate.

**This has to be further diluted with 4500 ml of water to have a working extraction buffer**

Store the unused portion of the working extraction buffer at 2 - 8° C. Thaw to room temperature before reuse.

**Preparation of working wash solution:**

Dissolve each packet of wash solution concentrate powder in 500 ml of distilled/deionised water and add 2.5 ml of Tween-20 which is provided along with the powder. Mix it well before use. The resultant wash solution is 10X concentrate..

**This has to be further diluted with 4500 ml of water to have a working wash solution**

Store the unused portion of the working wash solution at 2 - 8°C. Thaw to room temperature before reuse.

**Preparation of stop solution:**

Add 27.0 ml of 98 % $H_2SO_4$ in 973 ml of deionized or distilled water. Work in fume hood while preparing stop solution. Store at room temperature for up to one year.

**Preparation of user positive control:**

The user is advised to prepare in-house positive control. Crush or grind one known positive seed into a uniform powder and add 1.0 ml of extraction buffer. Wait for 10 minutes before use. Prepare fresh before use.

## Sample preparation:

**Individual seed:**

Crush or grind the seed thoroughly into a uniform powder using mortar and pestle or a seed crusher. Add 1.0 ml of extraction buffer to each sample. Mix well and allow the mixture to stand for 30-60 minutes at room temperature. Allow particles to settle and use only the supernatant to do the test. Wash and rinse grinding equipment carefully between samples to avoid cross contamination.

**Individual leaf:**

Weigh 20 mg (about two leaf punch) and put it in a microtube. Crush leaf with pestle in 0.5 ml working extraction buffer. Mix well and incubate for 30-60 minutes at room temperature. Allow particles to settle and use only the supernatant to do the test. Take precautions to avoid cross contamination between samples.

## Assay Procedure

Test protocol for individual seed or leaf samples. Estimated procedure time is 60 minutes.

1. Add 50 µl CP4EPSPS enzyme conjugate to each well.
2. Add 50 µl of working extraction buffer to blank well
   Add 50 µl of positive control in two wells and 50 µl of negative control in two wells.
   Add 50 µl of sample extracts to wells. Mix contents of the plate carefully, to avoid cross contamination.
3. Cover the plate and incubate for 45 minutes at room temperature.
4. Remove content of the wells by decanting into a sink or a waste container. Add 300 µl/well wash solution to all wells and then empty wells by inverting the plate. Repeat washing procedure three more times. Alternately, perform four washes by using microtiter plate washer. After last wash, tap the inverted microtiter plate on paper towel to remove as much liquid as possible.
5. Add 100 µl substrate per well.
6. Cover the plate and incubate for 15 minutes at room temperature.
7. Stop the reaction by adding 100 µl of stop solution.
8. Measure the absorbance at 450 nm (primary filter) and 620/630 nm (secondary filter). Read the plate within 15 minutes after addition of stop solution.

### Notes on technique:

1. Protect the plates from draught, strong light or direct sunlight during the test procedure.
2. Careful aspiration of the washing solution is essential for good assay precision.
3. Since the timing of the incubation steps is important to performance of the assay, use multi channel pipettes to dispense the sample/reagents.
4. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

## Interpretation of results:

### General test criteria:

- Absorbance value of the blank well should not exceed 0.1
- Absorbance value of blank should be subtracted from the absorbance values of positive control, negative control and samples.
- The coefficient of variance between the positive controls, in duplicate, should be less than 15 %
- Mean positive control should have absorbance of at least 0.5
- Mean negative control should have absorbance below 0.1. If above criteria are not met, the test is invalid and should be repeated.

## Qualitative test

### Cutoff Calculation for single cotton, maize or soybean leaf and seed samples:

Cutoff value = (Mean absorbance of negative control + 0.1)

### Positive or Negative Sample

Individual seed or leaf samples are either positive or negative.

**Positive sample:** If actual sample absorbance is more than the cutoff value.

**Negative sample:** If actual sample absorbance is less than the cutoff value.
Low level results normally indicate improper washing, cross contamination or technique. In such cases, retesting is recommended.

### Performance characteristics

Sensitivity: 100 %
Specificity: 100 %

## WARRANTY

Eurofins Amar Immunodiagnostics Pvt. Ltd. warrants that the products sold hereunder ("the Product") are defect-free in material and workmanship, provided they are used in accordance with the prescribed instructions before the expiry of the products as printed on the product label.

The customer should notify Eurofins Amar Immunodiagnostics in writing of Warranty defects during the warranty period, including an offer by the customer to return the Products to Eurofins Amar Immunodiagnostics for evaluation. Eurofins Amar Immunodiagnostics will repair or replace, at its sole option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Eurofins Amar Immunodiagnostics

## THIS WARRANTY IS EXCLUSIVE

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If any part of this Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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***Liberty Link is a register trademark of Bayer Crop Science.

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