Cry3Bb ELISA Kit

Cat No. AID 053

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Intended use

Cry3Bb ELISA test kit is intended to be used for the qualitative detection of Cry3Bb protein present in transgenic Maize plant carrying Monsanto Yieldgard Root worm RW event 863. The test is used to detect the Cry3Bb protein in individual Maize seed or leaf.

It can also be used to detect event 863 in bulk grain at 0.2% (1 positive in 500 negative seeds)

The total incubation time of the assay is 60 minutes for leaf/seed, and 90 minutes for bulk grain.

Principle of the test

An antibody specific to the Cry3Bb protein molecule is immobilized on the microwell plates and second antibody specific for Cry3Bb protein is conjugated with horse radish peroxidase (HRP). When the sample extracts are added to the microtiter wells, the Cry3Bb from sample binds to the antibody immobilized in the well. This binding is subsequently detected by addition of enzyme labeled antibody. After a washing step, substrate is added. The enzymatic reaction and development of color is proportional to the amount of Cry3Bb present in the sample. The reaction is terminated by addition of stopping solution. Absorbance is then measured on a plate reader. Light color indicates lower concentration while dark color indicates higher concentration of Cry3Bb protein in the sample.

Cross-reactivity

The Cry3Bb ELISA kit does not cross react with CP4EPSPS (Roundup Ready**), Cry1F, Cry1Ac, eCry3.1Ab (AgriSure Duracade), mCry3A, Cry1Ab, Cry2A, Vip3A, Cry34Ab1, Cry35Ab1 or PAT (LibertyLink***).

Precautions

The Cry3Bb ELISA test 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-Cry3Bb antibody, individually packed in a laminate bag with silica gel
2. Cry3Bb enzyme conjugate: One Ready- to- use bottle of 55 ml
3. Extraction buffer concentrate: One packet of powder with a 25 ml 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. Cry3Bb Negative control: One ready-to-use vial of 10 ml

Contents of 50-plate kit:

1. Fifty plates of 96 wells coated with Anti-Cry3Bb antibody, each packed individually in a laminate bag with silica gel
2. Cry3Bb enzyme conjugate: One ready to use bottle with 275 ml.
3. Extraction buffer concentrate: One packet of powder with 25 ml 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 500 ml
6. Cry3Bb Negative control: One ready-to-use vial of 10 ml

Material and equipment required but not provided

- Pipette with disposable plastic tips
- Multi channel pipette with disposable pipette tips
- Deionized or distilled water
- Graduated cylinders with one liter capacity
- Reagent troughs
- Plate ELISA washer or wash bottle
- Plate ELISA reader with 450/620 nm filter
- Table top centrifuge
- Analytical balance
- Marking pen, Parafilm*, Pipette tips, Timer and Paper towels.

Reagent preparation

The procedure for preparation of reagent is as follows

Preparation of working extraction buffer:

Dissolve the packet of extraction buffer 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 unused portion of working extraction buffer at 2 - 8° C. Thaw to room temperature before using the same again.

Preparation of Working wash buffer:

Dissolve the packet of wash buffer 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 needs to be further diluted with 4500 ml of water to have working wash buffer

Store unused portion of working wash buffer at 2 - 8° C. Thaw to room temperature before using the same again.

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 seed thoroughly into a uniform powder using mortar and grinder or seed crusher. Transfer it to a micro tube and add 1.0ml of extraction buffer. Allow the mixture to stand for 30-60 minutes at room temperature (RT). 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 micro tube. Crush leaf with pestle in 0.5 ml working extraction buffer. Mix well and incubate for 30-60 minutes at RT. Allow particles to settle and use only the supernatant to do the test. Avoid cross contamination between samples.

Test protocol 1 for individual seed or leaf samples. Estimated procedure time is 60 minutes

1. Allow all reagents to reach room temperature before use.
2. Add 50 µl enzyme conjugate to each well.

   Add 50 µl 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 it 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 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 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.

Test protocol 2 for bulk seed testing. Estimated procedure time is 90 minutes

1. Allow all reagents to reach room temperature before use.
2. Add 50 µl enzyme conjugate to each well.

   Add 50 µl 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 it for 60 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 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 30 minutes at room temperature.
7. Stop the reaction by adding 100 µl 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 of the blank well should not exceed 0.15
- Absorbance value of blank should be subtracted from positive control, negative control and samples.
- The coefficient of variance between the duplicate positive controls should be less than 15 %
- Mean positive control should have absorbance of at least 0.5
- Mean negative control should have absorbance below 0.2

If above criteria are not met, the test is invalid and should be repeated.

Qualitative test

For single corn leaf and seed samples:

Cutoff calculation for single corn leaf or seed samples :

Cutoff value = (Mean absorbance of negative control + 0.3)

Positive or Negative Sample

Individual seed or leaf samples are either positive or negative.

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

Negative sample: If 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 %

For bulk seed testing :

Sample is positive:

If the sample absorbance value is above 0.5, ground corn sample contains at least 1 positive Cry3Bb seed in 500 negative seeds.

Sample is negative:

If the sample absorbance value is below 0.5, ground corn sample does not contain 1 positive Cry3Bb seed in 500 negative seeds.

This is only a qualitative test.

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