Cry1Ac- Cry2A ELISA Kit

Cat No. AID 017

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Intended use

The Bollgard **II combination kit is used for simultaneous qualitative detection of Cry1Ac and Cry2A protein in cotton leaf or seed samples with both analytes, measured in the same well of the assay plate.

The format helps the laboratory to cut down work load by half thereby increasing productivity. The kit is ideally suited for testing large numbers of seed lots for Cry1Ac and Cry2A simultaneously.

Principle of the test

In the test, cotton sample extracts are added to test wells coated with antibodies raised against Cry1Ac and Cry2A proteins. Any Cry1Ac or Cry2A protein present in the sample extract binds to the antibodies. This is detected by addition of alkaline phosphatase - labeled Cry2A antibody or horseradish peroxidase - labeled Cry1Ac antibody. After a simple wash step, the results of the Cry2A assay are visualized via the addition of a pNPP substrate. Once the yellow color develops and is read, the wash step is repeated, and TMB substrate is added. The Cry1Ac results are visualized via the development of the resulting blue color which, subsequently, becomes yellow on addition of stop solution.

Cross-reactivity

The Cry1Ac - Cry2A ELISA Kit does not recognize CP4EPSPS (Roundup Ready**), Cry1F, Cry9C, CryBb, mCry3A, Cry1C or PAT (Liberty Link***).

Precautions

The Cry1Ac-Cry2A ELISA Kit is intended for in vitro use only. The reagents contain Thimerosal as a 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-Cry1Ac & Cry2A antibody, each packed individually in a laminate bag with silica gel
2. Cry1Ac & Cry2A combined 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. Substrate #1 (pNPP): One ready-to-use bottle of 110 ml
6. Substrate #2 (TMB): One ready-to-use bottle of 110 ml
7. Cry1Ac / Cry2A Positive control: One ready-to-use vial of 10 ml
8. Cry1Ac & Cry2A Negative control: One ready-to-use vial of 10 ml

Contents of 50-plate kit:

1. Fifty plates of 96 wells coated with Anti-Cry1Ac & Cry2A antibody, each packed individually in a laminate bag with silica gel
2. Cry1Ac & Cry2A combined conjugate: One ready – to - use bottle of 275 ml
3. Extraction buffer concentrate: One packet of powder with a 25ml bottle of Tween-20
4. Wash solution concentrate: One packet of powder with a 2.5 ml bottle of Tween-20
5. Substrate #1 (pNPP): One ready-to-use bottle of 500 ml
6. Substrate #2 (TMB): One ready-to-use bottle of 500 ml
7. Cry1Ac / Cry2A Positive control: One ready-to-use vial of 10 ml
8. Cry1Ac & Cry2A Negative control: One ready-to-use vial of 10 ml

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

Preparations of working wash solution

Dissolve the 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 working wash solution.

Store unused portion of 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 two hours (60+30+30 minutes)

1. Add 50 µl Cry1Ac-Cry2A enzyme conjugate in each well.
2. Add 50 µl of working extraction buffer in blank well.

   Add 50 µl of Cry1Ac-Cry2A positive control in two separate wells. Add 50 µl of Cry1Ac-Cry2A negative control in two separate wells. Add 50 µl of each sample to rest of the wells.
3. Incubate 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 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 100µl Substrate # 1.
6. Incubate for 30 minutes at room temperature.
7. Take absorbance at 405 nm with 630 nm as secondary filter. Presence of yellow color indicates that samples are positive for Cry2A.
8. Wash the plate in the same manner as indicated in step 4 above.
9. Add 100 µl Substrate # 2.
10. Incubate for 30 minutes at room temperature. Add 100 µl stop solution. Take absorbance at 450 nm with 630 nm as secondary filter. Presence of yellow color indicates that sample are positive for Cry1Ac.

Notes of Technique

1. Protect the plates from draught, strong light or direct sunlight during the testing procedure.
2. Careful aspiration of the working wash solution is essential for good assay precision.
3. Since the timing of the incubation steps is important for the performance of the assay, use multichannel 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 control, in duplicate, should be less than 15%.
- Each 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 leaf and seed samples is as follows:

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

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

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