Bt-Cry1Ac & Bt-Cry2A Multi-trait ELISA Kit

DAS ELISA for the detection of Bt-Cry1Ac & Bt-Cry2A proteins
Catalog number: PSM 14900

List of contents

Lot number	Item	480 wells	4800 wells
_____	Antibody-coated 96-well microtiter plates	5 strip	50 solid
_____	Alkaline phosphatase enzyme conjugate, concentrated	0.550 mL	1 x 5.5 mL
_____	Peroxidase enzyme conjugate, concentrated	0.550 mL	1 x 5.5 mL
_____	RUB6 enzyme conjugate diluent	55 mL	1 x 550 mL
_____	pNPP substrate solution, 1X	60 mL	550 mL
_____	TMB substrate solution	60 mL	550 mL
_____	Positive control	1	5
	The above items should be stored at 2 - 8 °C.		
_____	PBST wash buffer, 20X concentrate, liquid	7 x 50 mL	
_____	PBST wash buffer, powder		3 x 110 g
	The above item should be stored at room temperature (18 - 30 °C).		

Materials required but not provided

- Distilled water
- Paper towels
- Micropipette and Micropipette tips, sterile
- Seed extraction equipment.
    - Seed press or seed crusher and plate
    - Agdia sample mesh bag (ACC 00930) and rubber mallet
    - Graduated cylinder
    - Analytical balance
- Micro tubes and tube rack
- Airtight container for incubations (humid box)
- Plate reader with 405 nm and 650 nm filter

Storing the reagents

Store all kit components at the recommended temperature (above) to assure their full shelf life. Each ELISA plate pouch contains a desiccant packet. Keep the plate or unused testwells sealed in the pouch with the desiccant and store in the refrigerator (2 - 8 °C) between uses. Allow the components of the kit to warm to room temperature for about 30 minutes before use.

Concentrated enzyme conjugates or their diluted forms should not be mixed together and stored. Always prepare fresh antibodies the day you will perform the test.

Technical service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone 574-264-2014 or 800-622-4342 or by email (info@agdia.com).

Precautions

Prevent direct skin and eye contact with, or ingestion of, kit components. Obtain medical attention in case of accidental ingestion of kit components. It is recommended that gloves be worn while performing the assay. Always wash hands thoroughly after using the kit.

Intended Use

This dual-trait ELISA is intended for seed quality purposes to determine the presence or absence of the Bt-Cry1Ac & Bt-Cry2A proteins as expressed in transgenic cotton seed and leaf. The test does not distinguish between Bt-Cry1Ab and Bt-Cry1Ac proteins. This test shows no cross-reaction with Bt-Cry1F, CP4 EPSPS, PAT/bar, Vip3A, Bt-Cry1C or NPTII transgenic proteins.

Test Principle

The test system for Bt-Cry1Ac & Bt-Cry2A is a Double Antibody Sandwich (DAS) ELISA. Antibodies specific to Bt-Cry1A & Bt-Cry2A have been coated to the testwells of a microplate. Enzyme conjugate solutions have been included in this kit, containing monoclonal antibodies specific to Bt-Cry1A protein conjugated to horseradish peroxidase and monoclonal antibodies specific to Bt-Cry2A conjugated to alkaline phosphatase. The enzyme conjugate is added to the testwells followed by sample extracts. If either Bt-Cry1A or Bt-Cry2A protein is present in the sample, they are bound by the appropriate antibody and captured on the microplate.

After a short incubation the microplate is washed to remove any unbound enzyme conjugate and sample. The pNPP substrate is added to the microplate. If the alkaline phosphatase conjugate is present a color will be produced signifying the presence of Bt-Cry2A. The plate is washed and TMB substrate is added to the microplate. If the peroxidase conjugate is present a color will be produced signifying the presence of Bt-Cry1A. The color reactions can be measured with a spectrophotometer or observed visually.

Limitations

The following is a description of factors that could limit test performance or interfere with proper test results.

Samples: This test is only recommended for use with single seed and leaf samples of cotton.

Sample Extract Buffer: The Bt-Cry1Ac & Bt-Cry2A dual-trait ELISA must be used with 1X PBST for optimal results. Do not use sample extract buffers supplied with other ELISA kits.

Sample Dilution: ELISA performance is very dependent on the proper sample to buffer ratio (1:20 for seed - tissue weight in grams: buffer volume in mL and 1:10 for leaf – tissue weight in grams: buffer volume in mL).

Substrate solutions: Protect substrate solutions from light. Light or contamination could cause background color in negative wells.

Expiration: Test components expire one year from date of purchase. Negative controls, which are sold separately, expire one year from date of purchase.

Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly.

Stop solutions: Stop solutions should not be used with this test. Use of stop solutions will invalidate test results.

Preparing for the test

Familiarize yourself with the kit components and check that all components are present in the kit. Please read these instructions carefully before performing the test.

Prepare buffers

PBST is used as wash buffer and sample extraction buffer. PBST is supplied as either 20X concentrate or as a powder.

20X concentrate

Prepare 1X PBST wash buffer by diluting one 20X pouch of PBST wash buffer with 950 mL of distilled water.

powder

Prepare 1X buffer by dissolving PBST buffer powder in distilled water according to the table below:

Buffer powder	5 g
Distilled water	500 mL

Prepare controls

Positive and negative lyophilized controls can be reconstituted with 2.0 mL 1X PBST wash buffer per bottle.

Make control aliquots

After reconstituting the positive and negative control, divide them into aliquots, each sufficient for one use. Dispense aliquots into polypropylene tubes that can be securely capped. If you will be using a control in one well each time you run the test, prepare 120 µL aliquots. If you will be using a control in two wells, prepare 220 µL aliquots. Each aliquot should be sufficient for the tests to be run plus a small additional volume to assure easy dispensing.

Control aliquots must be stored frozen (-10 to -30 °C freezer or a non-frost-free household freezer). Do not thaw until just before use. At the time of each test run, remove from storage only the aliquots that will be used. Allow the tubes to thaw and mix the contents thoroughly. At the time you add sample extracts to testwells, add the same volume of negative and positive control to the appropriate control wells.

Do not subject the aliquots to multiple freeze-thaw cycles.

Prepare testwells

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping testwells in a humid box during incubation will help prevent samples from evaporating.

Make a copy of the loading diagram and record the locations of your samples and controls. We recommend that you use a buffer well, negative control well and positive control well on each plate each time you run the test.

Prepare Samples

Use the table below for quick reference when preparing samples for this assay.

TISSUE TYPE	SAMPLE TO BUFFER RATIO (tissue weight in g:buffer volume in mL)	EXTRACTION TIME	INCUBATION TIME	SUBSTRATE INCUBATION ALK PHOS	SUBSTRATE INCUBATION PEROX
SEED	1:20	3 minutes	60 minutes	30 minutes	20 minutes
LEAF	1:10	3 minutes	60 minutes	30 minutes	20 minutes

EXAMPLES

Seed at 1:20

- Add 6 mL of 1X PBST to a crushed seed weighing 0.3 g
- Massage/mix. Allow sample to extract for 3 minutes
- Assay 100 µL of the supernatant (upper liquid layer)

Leaf at 1:10

- Add 3 mL of 1X PBST to leaf material weighing 0.3 g
- Thoroughly macerate using clean mortar & pestle or Agdia mesh bag. Allow sample to extract for 3 minutes
- Assay 100 µL of the extract

Test Procedure

1. Prepare enzyme conjugate

Both bottles of the enzyme conjugate are concentrated (100X) and must be diluted with RUB6 enzyme conjugate diluent before use. Prior to use gently shake each vial 10 seconds or vortex for 5 seconds before using.

Add 110 µL of each bottle (alkaline phosphatase and peroxidase) of concentrated enzyme conjugate to 11 mL of RUB6 diluent; this will be sufficient for 1 plate.

Add 1.1 mL of each bottle (alkaline phosphatase and peroxidase) of concentrated enzyme conjugate to 110 mL of RUB6 diluent; this will be sufficient for 10 plates.

Mix the enzyme conjugate solution thoroughly before adding it to the plate.

Enzyme conjugates should not be mixed, either in concentrated form or diluted in RUB6, and stored. Prepare only as much enzyme conjugate as will be needed for one day.

2. Add enzyme conjugate

Dispense 100 µL of enzyme conjugate per well.

3. Dispense samples and controls

Following your loading diagram, dispense 100 µL of each prepared sample into the appropriate testwells of the ELISA plate. Add 100 µL of each positive and negative control into the appropriate testwell. Mix the contents of the wells by gently swirling the plate on the bench-top.

4. Incubate plate

Set the plate inside the humid box and incubate for 60 minutes at room temperature.

5. Wash plate

When the incubation with the sample and enzyme conjugate is complete, empty the testwells into a sink or waste container without allowing the contents of one testwell to mix with the contents of another testwell.

Fill all the testwells completely with 1X PBST, and quickly empty. Repeat 7 times. It is very important that all testwells are thoroughly washed. After washing, hold the plate upside down and tap firmly on a paper towel to remove any excess liquid.

Note: If using an automatic plate washer, please be sure that the machine is at the appropriate setting for washing flat bottom plates and at a wash volume of 300 µL per testwell.

6. Add pNPP substrate solution

Add 100 µL of pNPP substrate solution to each testwell.

Let the plate incubate for 30 minutes at room temperature. Keep testwells away from strong light.

7. Read at 405 nm (Bt-Cry2A detection)

Measure the optical density of the testwells on a plate reader at 405 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading.

Do not use a stop solution or make any subtractions from the O.D. values.

8. Evaluate Results

Visually

Wells in which a yellow color develops indicate positive results for the Bt-Cry2A trait. Wells in which there is no significant color development indicate negative results. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.

Plate Reader

Plate Validity: Test results are valid only if positive control wells are above 1.0 O.D. and buffer wells are less than 0.110 O.D.

Interpretation: Negatives should be rated ≤ 0.110 O.D. Positives should be rated at 0.500 O.D. or higher. Samples that obtain O.D. values greater than the buffer well value, but less than the positive O.D. threshold should be retested.

If either control well does not show the appropriate color, please repeat the test procedure. If the problem persists, contact Agdia for further assistance.

9. Wash plate

After recording the results for Bt-Cry2A, wash the plate 8 times with 1X PBST.

After washing, hold the plate upside down and tap firmly on a paper towel to remove any excess liquid.

10. Add TMB substrate solution

Add 100 µL of the TMB substrate solution into each well of the plate.

Let the plate incubate at room temperature for 20 minutes. Keep testwells away from strong light.

11. Read at 650 nm (Bt-Cry1A detection)

Measure the optical density of the testwells on a plate reader at 650 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading.

Do not use a stop solution or make any subtractions from the O.D. values.

12. Evaluate Results

Visually

Wells in which a significant blue color develops indicate positive results for the Bt-Cry1A trait. Wells in which there is no significant color development indicate negative results. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.

Plate Reader

Plate Validity: Test results are valid only if positive control wells are above 1.0 O.D. and buffer wells are less than 0.100 O.D.

Interpretation: Negatives should be rated ≤ 0.100 O.D. Positives should be rated at 0.500 O.D. or higher. Samples that obtain O.D. values greater than the buffer well value, but less than the positive O.D. threshold should be retested.

If either control well does not show the appropriate color, please repeat the test procedure. If the problem persists, contact Agdia for further assistance.

Buffer Formulations

PBST Buffer (Wash Buffer) (1X)

Dissolve in distilled water to 1000 mL:

Sodium chloride	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Potassium chloride	0.2 g
Tween-20	0.5 g

Adjust pH to 7.4

Date _____ Test _____

Test performed by _____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

agdia®