Bt-Cry3Bb1 ELISA Kit

DAS ELISA for the detection of Bt-Cry3Bb1 protein
Catalog number: PSP 06100

List of contents

Lot number	Item	480 wells	4800 wells
_____	Antibody–coated 96-well microtiter plates	5 strip	50 solid
_____	Peroxidase enzyme conjugate, concentrated	0.550 mL	1 X 5.5 mL
_____	RUB6 enzyme conjugate diluent	55 mL	1 X 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, powder or liquid	7	3 X 110 g
	The above items should be stored at room temperature (18 - 30 °C).		

Materials required, but not provided

Some of the items in the list below may be necessary depending on the type of samples and the method necessary to process the samples. Please refer to sample preparation section for guidance.

- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Airtight container for incubations
- Negative control (Catalog number: LNC 06100 – Please specify seed or leaf tissue when ordering.)
- Seed and leaf extraction equipment.
    - Seed press or seed crusher and plate
    - Agdia sample mesh bag (ACC 00930) and rubber mallet
    - Agdia sample mesh bag (ACC 00930) and marker with bag stand
    - Mortar and pestle
    - Micro tube and pestle with tube rack
- Graduated cylinder
- Analytical Balance
- Micro tubes and tube rack
- Plate reader with 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 using.

Once the concentrated enzyme conjugate has been diluted to 1X in RUB6, it can be stored for 2 weeks in the refrigerator.

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, product 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 this product.

Please read these instructions carefully before performing the test.

Intended Use

This kit is intended for seed quality purposes to determine the presence of the Bt-Cry3Bb1 protein in YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ corn hybrids. Using this test system, you can reliably confirm the presence of Bt-Cry3Bb1 protein in single leaves and single seeds.

This test recognizes Bt-Cry3Bb1 protein and does not differentiate between and shows no cross-reaction with Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry1F, Bt-Cry2A, Bt-Cry3A, Bt-Cry9C, Bt-Cry34Ab1, Bt-Cry35Ab1, NPTII, EPSPS (events GA21 and NK603), or PAT (Liberty Link®) proteins. This kit does not differentiate between the Bt-Cry3Bb1 proteins produced in YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ corn hybrids.

YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2, YieldGard VT Triple® are registered trademarks of Monsanto Company.

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Test Principle

The test system for Bt-Cry3Bb1 is a direct Double Antibody Sandwich (DAS) ELISA. Polyclonal antibodies specific to Bt-Cry3Bb1 have been coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit, containing monoclonal antibodies specific to Bt-Cry3Bb1 protein conjugated to a peroxidase enzyme. Enzyme conjugate is added to the testwells followed by sample extracts. If Bt-Cry3Bb1 protein is present in the sample, it is bound by the antibodies and captured on the microplate.

After a short incubation, the microplate is washed to remove any unbound enzyme conjugate and sample. The TMB substrate is added to the microplate. If the peroxidase conjugate is present, a color will be produced signifying the presence of Bt-Cry3Bb1 protein. The color reaction must be measured using a spectrophotometer and results interpreted.

Limitations

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

Expiration: Test components 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.

Samples: This test has been validated in corn only.

Sample Extract Buffer: The Bt-Cry3Bb1 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. Samples should be extracted at the recommended tissue to buffer ratio listed in these instructions.

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

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

Prepare only the amount of 1X buffers needed for the day.

PBST wash buffer

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

Reconstitute lyophilized positive control and lyophilized negative control with 2.0 mL 1X PBST wash buffer per bottle.

Make control aliquots

After preparing the positive and negative control, divide them into aliquots, each sufficient for one use. Dispense aliquots into 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 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 refreeze controls.

Prepare testwells

If you will be using less than a full 96-well plate, remove any unused strips and seal them in the foil pouch with the desiccant. Using a permanent marker, number the strips in case a strip becomes separated from the frame.

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.

Grind and dilute samples

Leaves, seedlings, or seeds must be ground and diluted in 1X PBST buffer.

Leaf Samples

Grind leaf in 1X PBST wash buffer at a ratio of 1:10 (tissue weight in g: buffer volume in mL). Leaves can be ground in Agdia mesh sample bags (ACC 00930) or with a mortar and pestle. If using a mortar and pestle be sure to wash and rinse it between samples. Allow the sample to extract for at least 3 minutes at room temperature.

Seed Samples	Weigh and record the average weight of each sample. Thoroughly crush seeds into a uniform powder. Single seeds can be crushed in a seed crusher or with a hammer. Wash and rinse the grinding equipment between samples. Dilute the crushed seed sample in 1X PBST buffer at a ratio of 1:10 (tissue weight in g: buffer volume in mL), typically 1 seed in 3 mL of buffer. Mix the seed powder and buffer, and let stand for at least 3 minutes at room temperature. Use only the supernatant (liquid layer) when adding sample extracts to testwells.

Test Procedure

1. Prepare enzyme conjugate	The enzyme conjugate is 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 concentrated enzyme conjugate to 11 mL of RUB6 diluent, this will be sufficient for 1 plate.* *Add 1.1 mL of concentrated enzyme conjugate to 110 mL of RUB6 diluent, this will be sufficient for 10 plates.* Mix the enzyme conjugate thoroughly before adding it to the plate. Any unused conjugate must be stored in the refrigerator and used within two weeks of diluting.
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 2 hours at room temperature or overnight in the refrigerator.
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 TMB substrate solution	Add 100 µL of the TMB substrate solution into each well of the plate.
7. Incubate plate	Incubate the plate for 20 minutes. Keep testwells away from strong light.

8. Evaluate results

Measure the optical density of the testwells on a plate reader at 650 nm or visually. Wells in which a blue color develops indicate positive results. 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.

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 _____

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A												
B												
C												
D												
E												
F												
G												
H