Bt-Cry3A ELISA

PathoScreen kit for Bt-Cry3A endotoxin in plants
Catalog number: PSA 05900

Contents List

Lot number	Item	96 wells	288 wells	480 wells
_____	Antibody-coated 96-well microtiter plates	1 strip	3 strip	5 strip
_____	Alkaline phosphatase enzyme conjugate	0.150 ml	0.350 ml	0.550 ml
_____	PNP substrate tablets, 5 mg each	12	12	25
_____	PNP substrate buffer, 5X concentrate	12 ml	12 ml	25 ml
_____	Nonfat dried milk	5 g	5 g	2 X 5 g
_____	Positive control	1	1	1
_____	Negative control	Sold separately	Sold separately	Sold separately
	The above items should be stored at 4° C.			
_____	PBST wash buffer	3	5	7
_____	Tween 20	15 ml	30 ml	30 ml
	The above items should be stored at room temperature.			

Materials required, but not provided

- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Lyophilized negative control can be purchased from Agdia
- Sample grinding device such as:
    - Agdia sample mesh bag (ACC 00930)
    - Agdia tissue homogenizer (ACC 00900)
    - Mortar and pestle
- Airtight container for incubations

Limitations

Expiration: This test should be used within 1 year of purchase.

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

Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use. Buffer formulations on page 5 are for reference only.

Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Precautions

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components. Always wash hands thoroughly after using this product.

Preparing for the test

Familiarize yourself with the kit components. Check that all components are present in the kit.

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Intended use

Bt endotoxins are proteins produced in plants or in culture expressed by genes taken from the bacterium, *Bacillus thuringiensis.* Using this test system, you can detect Bt endotoxin extracted from transgenic potato sprouts, tubers and leaves expressing the truncated Cry3A gene.

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).

Prepare buffers

Prepare only as much 1X buffers that will be needed for one day.

PBST wash buffer

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

MEB extraction buffer (1X)

Dissolve in 100 ml 1X PBST:

Tween-20	0.5 g
Nonfat dried milk	0.4 g

Stir for 30 minutes. Adjust pH to 7.4. This buffer contains nonfat dried milk as a source of protein for blocking. Prepare only as much of this buffer as you will need for one day.

ECM buffer (1X)

Dissolve in 25 ml 1X PBST:

Nonfat dried milk	0.1 g

Stir for 30 minutes. Adjust pH to 7.4. This buffer contains nonfat dried milk as a source of protein for blocking. Prepare only as much of this buffer as you will need for one day.

PNP substrate buffer (1X)

PNP substrate buffer is used to dilute PNP substrate tablets. The volume of 1X PNP buffer required depends on the number of testwells used. You will need 100 µl of prepared PNP substrate buffer for each testwell you are using. To estimate the volume needed, prepare 1 ml for each 8 well strip used. A full plate will require about 10 ml.

To prepare 10 ml of working PNP substrate buffer, mix 2 ml of 5X PNP buffer concentrate with 8 ml of distilled water.

Note: PNP substrate tablets will not be added at this time. Tablets will be added prior to completion of the enzyme conjugate incubation.

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 and positive control well on each plate each time you run the test.

Prepare controls

If you are running a qualitative assay reconstitute the bottle of lyophilized positive control and negative control with 2 ml MEB sample extraction buffer. The reconstituted positive control will have a concentration of about 25 ng/ml.

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 (-20°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.

Grind and dilute samples

Sprouts, tubers, stems and leaves can be used for testing. Kept in mind, too many plant samples per well can reduce the sensitivity of the test.

Tubers Potato tubers can be tested individually or composite samples can be made. Use no more than 4 tubers per composite. Using a knife or peeler, slice a thin area of the skin from the tuber. It is best to take skin from the ends of the tuber. Cut the skin as thin as possible (1mm thick). Grind the skin sample with the MEB sample extraction buffer. Generally, skin samples require dilutions of 1:2 (weight in g: buffer volume in ml).

Leaves and sprouts Grind samples with MEB buffer in sample extraction bags or by mortar and pestle. Some plant parts will produce very high concentrations of Bt endotoxin in the sample extracts. Generally, leaves require a tissue to buffer ratio of 1:10, dilutions up to 1:100 (weight in g: buffer volume in ml) are possible depending on the expression. This is best determined by experiment for the particular plants that are being tested.

Note: Always sanitize cutting equipment between samples to prevent possible contamination. If you are using a knife or razor blades to obtain samples, disinfect the cutting area and sampling equipment with a 1:2 bleach solution between each sample.

Prepare MEB extraction buffer.

Grind plant tissue in MEB extraction buffer at suggested ratio (tissue weight in g: buffer volume in ml) per tissue type. You will need 100 µl of diluted sample extract per testwell, plus an additional amount to assure easy dispensing. You can use Agdia's sample mesh bags (ACC 00930), Agdia's tissue homogenizer (ACC 00900) or other grinding devices to grind samples.

Test Procedure

1. Dispense samples

Following your loading diagram, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of sample extraction buffer into buffer wells.

2. Incubate plate

Set the plate inside the humid box and incubate for 1 hour at room temperature.

3. Prepare enzyme conjugate	**Note: Always prepare enzyme conjugate within 10 minutes before use. All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polystyrene, polypropylene or polycarbonate.** The bottle of alkaline phosphatase enzyme conjugate is supplied as a concentrate and must be diluted with ECM buffer before use. The recommended conjugate to buffer ratio is given on the label. Dispense the appropriate volume of prepared ECM buffer into a dedicated container. You will need 100 µl of buffer for each testwell you are using. Then, add the alkaline phosphatase enzyme conjugate according to the dilution given on the labels. *Example: If the dilution given on bottle of concentrated alkaline phosphatase enzyme conjugate is 1:100, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml ECM buffer. Then, add 100 µl of the concentrated enzyme conjugate to the ECM buffer.* After adding the enzyme conjugate, mix thoroughly. It is important to mix the enzyme conjugate solution well.
4. Wash plate	When the sample incubation is complete, wash the plate. Use a quick flipping motion to dump the wells into a sink or waste container without mixing the contents. Fill all the wells completely with 1X PBST, then quickly empty them again. Repeat 7 times. Hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.
5. Add enzyme conjugate	Dispense 100 µl of prepared enzyme conjugate per well.
6. Incubate plate	Set the plate inside the humid box and incubate for 1 hour at room temperature.
7. Prepare PNP solution	Each PNP tablet (ACC 00404) will make 5 ml of PNP solution, at a concentration of 1 mg/ml, about enough for five 8-well strips. About 15 minutes before the end of the above incubation step, measure 5 ml of room temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the buffer. **Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.**
8. Wash plate	As before, wash the plate 8 times with 1X PBST. Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.
9. Add PNP solution	Dispense 100 µl of PNP substrate into each testwell.
10. Incubate plate	Incubate the plate for 60 minutes. Plates should be protected from direct or intense light.

11. Evaluate results

Examine the wells by eye, or measure 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.

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

Results may be interpreted after more than 60 minutes of incubation as long as negative wells remain virtually clear.

Buffer Formulations

PBST 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

PNP substrate buffer (1X)

Dissolve in 800 ml distilled water:

Magnesium chloride hexahydrate	0.1 g
Sodium azide	0.2 g
Diethanolamine	97.0 ml

Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C.

Date _____ Test _____

Test performed by _____

	1	2	3	4	5	6	7	8	9	10	11	12
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B												
C												
D												
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