Bt-Cry2A ELISA Kit

Qualitative DAS ELISA for the detection of the Bt-Cry2A transgenic protein
Catalog number: PSP 05801

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	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 (Agdia catalog number: LNC 05801 - *Please specify leaf or seed control when ordering*.)
- Scissors, marker, timer
- Single seed and leaf extraction equipment.
    - Seed press or seed crusher and plate
    - Agdia sample mesh bag (ACC 00930) and rubber mallet or marker with bag stand
    - Mortar and pestle
    - Micro tube and pestle with tube rack
- Graduated cylinder
- Analytical balance
- Microtubes and tube rack

Storing the reagents

Store all kit components at the recommended temperature 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.

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.

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Intended Use

This ELISA was developed for the qualitative detection of the Bt-Cry2A protein in corn leaf or seed. This ELISA is validated to detect the Bt-Cry2Ab protein expressed from Monsanto's transgenic corn event, MON89034.

The Bt-Cry2A antibody recognizes Bt-Cry2A protein and shows no cross-reaction with Bt-Cry3Bb1, Bt-Cry34Ab, Bt-Cry1Ab, Bt-Cry1Ac, Bt-Cry1F, CP4 EPSPS (Roundup Ready®), GA21, NPT II, mBt-Cry3A (Agrisure ™ RW) or PAT (Liberty Link®).

LibertyLink® is a registered trademark of Bayer CropScience
Roundup Ready® is a registered trademark of Monsanto Company
Agrisure™ RW is a registered trademark of Syngenta Group Company

Test Principle

The test system for Bt-Cry2A is a direct DAS ELISA. Monoclonal antibodies specific to Bt-Cry2A are coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit containing monoclonal antibodies specific to Bt-Cry2A conjugated to a peroxidase enzyme. Enzyme conjugate is added to the testwells followed by sample extracts. If Bt-Cry2A is present in the sample, it is bound by the antibodies and captured on the microplate. The plate is then washed to remove any unbound enzyme conjugate and sample. Finally, a substrate is added to the microplate. If peroxidase is present, a color will be produced signifying the presence of Bt-Cry2A. The color reaction can be measured with a plate reader or observed visually.

Please read these instructions carefully before performing the test.

Limitations

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

Buffers: Prepare only the amount of 1X buffers needed for the day. Dilute only the amount of enzyme conjugate necessary at the time of each test run. Do not store 1X buffers.

Samples: This test has been evaluated in corn only.

Sample Extraction Buffer: The Bt-Cry2A ELISA must be used with 1X PBST wash buffer for optimal results. Do not use sample extraction buffers used with other ELISA kits.

Sample Dilution: ELISA performance is very dependent on the proper sample dilution (tissue weight in g: buffer volume in mL).

Expiration: Test should be used within one year of purchase.

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

Timing: Please follow times provided for extraction and incubation. Timings for each sample type have been optimized to give the best results for both negative and positive samples. **Not adhering to these exact times will interfere with achieving proper test results.**

Preparing for the test

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

PBST wash buffer

PBST wash buffer has been provided in either liquid or powder form. Depending upon your kit size, prepare according to directions as follows:

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 PBST wash 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 of prepared 1X PBST sample extraction 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.

Preparing Single Leaf and Seed Samples

Leaves, seedlings, or seeds must be ground and diluted in 1X PBST sample extraction buffer. For best results, samples should be diluted in 1X PBST buffer according to the ratios and times listed in the table below.

Individual leaves

Sample grinding in Agdia sample mesh bags.

For leaf samples use Agdia's sample mesh bags, a clean mortar and pestle, or any other grinding device that can break up leaf tissues and prevent contamination between samples.

A simple method for grinding a single leaf sample is by using Agdia's sample mesh bags. Use only one sample per bag and be sure to label each bag. Place two leaf punches between the mesh linings of the bag. Add 600 µL of 1X PBST buffer. Rub the bag with a marker to completely crush the sample. Massage the bag by hand for a few seconds to ensure good extraction. Let the extract sit for 3-5 minutes.

Single seeds

Single seeds can be crushed in a seed press, seed crusher or sample mesh bag and rubber mallet. Wash and rinse the grinding equipment between samples.

Crush seed and add 600 µL of 1X PBST buffer. Mix, on a rotary shaker, for 15 minutes to ensure good extraction.

Test Procedure

1. Prepare enzyme conjugate

Note: Always prepare enzyme conjugate within 10 minutes before use.

The enzyme conjugate is supplied as a concentrate and must be diluted with RUB6 diluent before use. The total amount of RUB6 diluent required depends on the number of testwells used. You will need 100 µL of buffer for each testwell you are using, plus an additional amount to assure easy dispensing. Dilute only the amount of enzyme conjugate needed for your test run.

Example: If the dilution given on the bottle of concentrated enzyme conjugate is 1:100, and you are preparing 10 mL of enzyme conjugate solution, you should first dispense 10 mL RUB6 diluent. Then, add 100 µL of the concentrated enzyme conjugate to the RUB6 diluent.

After adding the enzyme conjugate, mix thoroughly. It is important to mix the enzyme conjugate solution well.

2. Add enzyme conjugate

Dispense 100 µL of enzyme conjugate per well.

3. Dispense samples, controls, and buffer

Following your loading diagram, dispense 100 µL of each prepared sample into sample wells. Dispense 100 µL of positive control into the positive control wells, 100 µL of negative control into the negative control wells, and 100 µL of 1X PBST buffer into the buffer wells.

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. Warm TMB substrate

About 15 minutes before the end of the above incubation step, measure the required amount of TMB substrate needed. Return the remaining TMB substrate to the refrigerator. Allow measured TMB substrate to warm to room temperature. Caution: The TMB substrate is light sensitive, extra precautions are necessary to protect it from light sources when warming to room temperature.

You will need 100 µL of substrate for each testwell you are using. To estimate the volume needed, measure 1 mL for each 8 well strip used. A full plate will require about 10 mL.

6. 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, and then quickly empty them again. Repeat 7 times.

After washing, hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.

Note: If using an automatic plate washer, please be sure that the machine is at the appropriate settings for washing flat bottom plates.

7. Add TMB substrate

Add 100 µL of the TMB substrate solution into each well of the plate.
Let the plate incubate for 20 minutes. Keep testwells away from strong light.

8. Evaluate results

Measure O.D.'s 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.

For the test to be valid the following criteria must be met:

- The positive control value must be greater than or equal to 1.0 O.D.
- The positive leaf or seed value must be greater than 1.5 O.D.
- The negative control or buffer value must be less than or equal to 0.1 O.D.

O. D. values are based on raw data with no blanking or subtraction of negative values. Stop solutions cannot be used with this test and O. D. values obtained from stopping the reaction cannot be applied to this criterion**.**

If either control well does not give the appropriate O.D. value, please repeat the test procedure. If problems persist, please contact Agdia for further assistance.

Buffer Formulations

The following buffer is a standard part of your kit. This formulation is for reference only.

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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