Bt-Cry34Ab1 ELISA Kit

Quantitative DAS ELISA for the detection of the Bt-Cry34Ab1 transgenic protein
Catalog number: PSP 11500

List of contents

Lot number	Item	96 wells	288 wells	480 wells
_____	Antibody-coated 96-well microtiter plates	1	3	5
_____	Peroxidase enzyme conjugate, concentrated	0.150 mL	0.350 mL	0.550 mL
_____	RUB6, enzyme conjugate diluent	11 mL	33 mL	55 mL
_____	TMB substrate solution	25 mL	40 mL	60 mL
_____	Positive control	1	1	1
	The above items should be stored at 2 - 8 °C			
_____	PBST buffer	3	5	7
	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
- Scissors, marker, timer
- Additional sample extraction buffer (PBST Agdia catalog number: ACC 00501) will be required if most of the samples tested are grain samples.
- 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
- Supplemental full grind sampling equipment.
  - Blender and accessories
    - Blender (at least 450 watts)—optimal results were obtained using an Osterizer® blender at high speed (Sunbeam Corporation Model Number 6641, 1-800-597-5978)
    - Blender jars 125mL, Nalgene (“Mason” type, Fisher Scientific Catalog Number 11-815-10C)
    - Blender blade pack assembly (Oster® Sunbeam Product Catalog Number 4961)
    - Threaded bottom cap (Oster® Sunbeam Product Catalog Number 4902)

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.

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Safety

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

Intended Use

This kit has been validated and approved by Dow AgroSciences® for the quantitative analysis of the HERCULEX® RW trait.

HERCULEX® RW is a registered trademark of Dow AgroSciences LLC.

Test Principle

The test system for Bt-Cry34Ab1 is a direct DAS ELISA. Polyclonal antibodies specific to Bt-Cry34Ab1 are coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit containing monoclonal antibodies specific to Bt-Cry34Ab1 conjugated to a peroxidase enzyme. Enzyme conjugate is added to the testwells followed by sample extracts. If Bt-Cry34Ab1 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-Cry34Ab1. 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-Cry34Ab1 ELISA must be used with 1X PBST 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 1 year from date of purchase.

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

Timing: Please follow as closely as possible the recommended incubation times. Timings have been optimized to give the best results for both negative and positive samples. **Note: Please follow tables provided for extraction and incubation times. Not adhering to these exact times will interfere with achieving proper test results.**

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).

Preparing for the test

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

Prepare buffer

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

20X concentrate

Prepare PBST buffer by diluting one 20X pouch of PBST 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

Hydrating control & Preparing standard curve

It is recommended that these controls be used only once. Do not aliquot and refreeze.

Hydrate the control with 2 mL of 1X PBST, recap and thoroughly mix by shaking or vortexing. Be sure to invert the bottle with the cap on to ensure that any protein attached to the cap during lyophilization has a chance to solubilize. After hydration, begin preparing your standard curve. It is recommended that you produce a five point curve starting at 10 ng/mL and dilute to 0.625 ng/mL.

All points in the standard curve should be plated at least in duplicate for the test to be valid. For more accurate results it is recommended to perform this 1:1 dilution outside of the testwell in an appropriate micro dilution tube using a minimum of 400 µL of total transfer per concentration.

Prepare testwells

All samples to be tested should be plated at least in duplicate for the test to be valid.

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 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.

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 the leaf sample between the mesh linings of the bag. Rub the bag with a marker to completely crush the sample and to mix the contents uniformly. Add the appropriate volume of 1X PBST buffer to the bag containing the crushed leaf following a 1:50 (tissue weight in g: buffer volume in mL) leaf to buffer ratio and massage the bag by hand for a few seconds to ensure good extraction. Let the extract sit for 3 minutes before the next dilution in 1X PBST at 1/20. Transfer diluted sample to the testwells of the ELISA plate.

Leaf Tissue	Sample to buffer ratio	Extraction Time
1st dilution	1:50 g:mL	3 minutes
2nd dilution	1:20 mL:mL	0 minutes
Example		
1st dilution 1:50 • Add 3 mL of 1X PBST to a ground leaf sample weighing 0.06 g. • Mix and allow the extract to sit for 3 minutes. 2nd dilution 1:20 • Transfer 0.5 mL of extract to a tube containing 9.5 mL of 1X PBST.		

Seeds

Single seed
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.

Determine the weight of the seed. Crush and add the appropriate volume of 1X PBST buffer following a 1:50 (tissue weight in g: buffer volume in mL) seed to buffer ratio. Mix or massage for a few seconds to ensure good extraction. Let the extract sit for 3 minutes before the next dilution in 1X PBST at 1/30. Transfer diluted sample to the testwells of the ELISA plate.

Fully ground seed
Put the seed sample (minimum of 25 seeds) in a dry "Mason" jar and assemble the blade attachment. A 125 mL jar is recommended for 25 seeds. Grind the seed at high speed for about 60 seconds. Remove the jar from the blender and tap to collect all the powder. Shake the jar to mix and check for any unground seeds. Weigh a determined amount of ground seed. Extract and test as for crushed single seed.

Cleaning:
It is very important that the grinding equipment and workspace is cleaned well between each sample extraction. Wash blades, threaded caps and jars with detergent making sure all ground material is washed away. Be especially careful to clean crevices of the blade. Any remaining powder can contaminate the next sample.

Grinding:
Seed grinding guidelines described in this instruction are optimized for an Osterizer® blender with a power rating of 450 watts. Blenders of lower power may require a longer grinding time. Other devices like coffee grinders or ball mills may also be used to grind the seeds. Visually check that all seed has been ground to a fine powder.

Seed Tissue	Sample to buffer ratio	Extraction Time
1st dilution	1:50 g:mL	3 minutes
2nd dilution	1:30 mL:mL	0 minutes
Example		
1st dilution 1:50 • Add 15 mL of 1X PBST to a ground seed weighing 0.30 g • Mix and allow the extract to sit for 3 minutes. 2nd dilution 1:30 • Transfer 0.5 mL of extract to a tube containing 14.5 mL of 1X PBST.		

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. Dilute only what is needed for one day.

Add 110 µL of concentrated enzyme conjugate to 11 mL of RUB6 diluent, this will be sufficient for 1 plate.

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

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 at room temperature. Use the table to determine appropriate time for sample type.

Leaf Sample	Seed Sample
60 minutes	60 minutes

5. Warm TMB substrate solution

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: 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 solution

Add 100 µL of the TMB substrate solution into each well of the plate. Let the plate incubate according to the table below. Assure testwells are protected from strong light.

Leaf Sample	Seed Sample
30 minutes	30 minutes

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.

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.

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

Optional: Stop solution may be added to terminate the peroxidase/TMB reaction. Add 1N HCl at 100 µL per testwell. Read at 450 nm up to one hour after addition. Use of the stop solution may require adjustment of the points on the standard curve or further dilution of the test sample.

9. Analyze Results

Replicate OD readings at each point of the standard curve should be averaged and the results analyzed using a second order polynomial fit. The concentration of the Bt-Cry34AB1 trait can then be calculated by referencing this curve.

NOTE: If the R^2 value is below 0.9970, this could skew the ng/mL protein calculated value.

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

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Date _____ Test _____

Test performed by _____

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