CspB ELISA Kit

DAS ELISA for the detection of CspB protein
Catalog number: PSP 35500

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, 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 or purified water
- Paper towels
- Micropipette and Micropipette tips
- Leaf extraction equipment.
    - Agdia sample mesh bag (ACC 00930) and marker with bag stand
    - Mortar and pestle
    - Graduated cylinder
    - Analytical balance
- Micro tubes and tube rack
- Airtight container for incubations (humid box)
- Plate reader with 650 nm filter

Storing the reagents

Store all kit components at the recommended temperature to ensure 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.

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 ELISA was developed for the qualitative detection of the CspB protein in corn leaf. This ELISA is validated to detect the CspB protein expressed from Monsanto's transgenic corn event MON87460. When testing leaf, Agdia recommends testing coleoptile leaf tissue when possible. Lower OD values are expected when testing leaf material beyond the coleoptile stage. This test shows no cross-reaction with Bt-Cry1Ab, Bt-Cry1A.105, Bt-Cry2A, Bt-Cry3Bb1, NPTII, or CP4 EPSPS transgenic proteins.

Test Principle

The test system for CspB is a Double Antibody Sandwich (DAS) ELISA. Antibodies specific to CspB have been coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit containing monoclonal antibodies specific to CspB protein conjugated to horseradish peroxidase. The enzyme conjugate is added to the testwells followed by sample extracts. If CspB protein is present in the sample, it is 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. A TMB substrate is added to the microplate. If the peroxidase conjugate is present a color will be produced signifying the presence of CspB. 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 leaf samples of corn.

Sample Extract Buffer: The CspB 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 (1:10 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.

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. 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 ensure 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 PEROX**
LEAF	1:10	3 minutes	60 minutes	30 minutes

EXAMPLE

Leaf at 1:10

- Add 3.0 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

The enzyme conjugate is supplied as a concentrate (100X) and must be diluted with RUB6 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 solution thoroughly before adding it to the plate.

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

Leaf Sample
60 minutes

5. TMB substrate solution

About 15 minutes before the end of the above incubation step, measure the required amount of TMB substrate. Return the remaining TMB substrate to the refrigerator. Allow measured TMB substrate to warm to room temperature. Caution: TMB substrate is light sensitive. Protect it from light sources.

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 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.

7. Add TMB substrate solution

Add 100 µL of TMB substrate solution to each testwell.

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

8. Read at 650 nm

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.

9. Evaluate Results

Visually

Wells in which a blue color develops indicate positive results for the CspB 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: Data gathered from validation tests performed by several operators on a variety of corn lines was used to determine the following positive and negative cutoff O.D. values.

Optical Density	Test Result
Greater than 0.5	Positive
Less than 0.1	Negative
Between 0.1 – 0.5	Indeterminate result, requires more analysis

To interpret samples in the indeterminate OD range and to perform a more discriminating analysis of your data, perform the following analysis:

Compute the average of obtained buffer well O.D. values. Using this obtained average value, multiply by 5. Values greater than the value obtained should be interpreted as positive

Example:

Buffer Well 1 = 0.088 O.D Buffer Well 2 = 0.086 O.D.
Average (0.088, 0.086) = 0.087 O.D.
Positive O.D. Cutoff ≥ (0.087 x 5) = 0.435

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®