Vip3A ELISA Kit

DAS ELISA for the detection of Vip3A protein
Catalog number: PSP 83500

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
- Negative control (Agdia catalog number: LNC 83500 – *please specify leaf or seed control when ordering*.)
- Seed extraction equipment.
    - Seed press or seed crusher and plate
    - Agdia sample mesh bag (ACC 00930) and rubber mallet
    - Graduated cylinder
    - Analytical balance
- Grinding equipment for composite testing:
    - Blender (Osterizer® Sunbeam Corporation, Model No.,1-800-597-5978)
    - Blender jars 1000 mL, Nalgene (“Mason” type, Fisher Scientific, Catalog No. 2115-1000)
    - Blender blade pack assembly (Factory Services Inc., Catalog No. OC-DUX, 1-800-237-8699)
    - Threaded bottom cap (Factory Services Inc., Catalog No. OJN)
- Airtight container for incubations (humid box)
- Micro tubes and tube rack
- Plate reader with 650 nm filter

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 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 Vip3A protein in corn leaf or seed. This ELISA is validated to detect the Vip3A protein expressed in commercial products containing Syngenta's transgenic corn event MIR162 (Agrisure Viptera®). This test shows no cross-reaction with Bt-Cry1Ab, Bt-Cry1A.105, Bt-Cry2A, mBt-Cry3A, Bt-Cry1F, Bt-Cry3Bb1, Bt-Cry34/35Ab1, ecry3.1Ab, CspB, amy797E, PAT/pat, NPTII, GA21 or CP4 EPSPS transgenic proteins.

Test Principle

The test system for Vip3A is a Double Antibody Sandwich (DAS) ELISA. Antibodies specific to Vip3A have been coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit containing monoclonal antibodies specific to Vip3A protein conjugated to horseradish peroxidase. The enzyme conjugate is added to the testwells followed by sample extracts. If Vip3A 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 Vip3A. 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 seed and leaf samples of corn.

Sample Extract Buffer: The Vip3A 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 seed: 1.0 mL 1X PSBT buffer - 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 assure 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**
SEED	1 seed: 1.0 mL	3 minutes	60 minutes	30 minutes
LEAF	1:10	3 minutes	60 minutes	30 minutes

EXAMPLES

Seed at 1 seed: 1.0 mL 1X PSBT

- Add 1.0 mL of 1X PBST to a crushed seed
- Massage/mix. Allow sample to extract for 3 minutes
- Assay 100 µL of the supernatant (upper liquid layer)

Leaf at 1:10

- Add 3 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 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 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

Data gathered from validation tests performed by several operators in different labs on a variety of corn lines was used to determine the following positive and negative cutoff O.D. values.

Optical Density	**Test Result**
LPC Greater than 1.0	Positive
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:

Sort all of the data from a single microplate into a series of increasing OD values. Plot the OD values either as a histogram or x, y scatter plot with no x axis input. From the plots determine visually the OD value of the high end of the apparent negative sample population. Compute the average [Avg] and standard deviation [SD] for the apparent negative population from the sorted data.

Then, positive sample OD (ODpos) should be > [Avg] + 4 X [SD].

After computing an ODpos threshold, check the ODpos determined above for consistency with the generated histogram and with known samples.

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
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