eCry3.1Ab ELISA Kit

DAS ELISA for the detection of eCry3.1Ab protein
Catalog number: PSP 49000

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 refrigerated (2 - 8 °C)		
_____	PBST wash buffer, 20X concentrate, liquid	7 x 50 mL	
_____	PBST wash buffer, powder		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 and Micropipette tips
- Airtight container for incubations (humid box)
- 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
    - Agdia tissue homogenizer (ACC 00900)
    - Graduated cylinder
    - Analytical balance
    - Micro tubes and tube rack
- Grain sampling equipment.
    - Balance 1 - 500 g
    - Blender and accessories
        - Blender (at least 450 watts)—optimal results were obtained using an Osterizer® blender at high speed
        - Blender jars 250 mL, Nalgene (“Mason” type, Fisher Scientific Catalog Number 11-815-10d
        - Blender blade pack assembly (Oster® Sunbeam Product Catalog Number 4961)
        - Threaded bottom jar base (Oster® Sunbeam Product Catalog Number 4902)
- Plate reader with 650 nm filter

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 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 when handling the enzyme conjugate solution. Always wash hands thoroughly after using the kit.

Intended Use

This ELISA was developed to detect the presence or the absence of the eCry3.1Ab protein expressed in corn seed, leaf or grain. This ELISA is validated to detect the eCry3.1Ab protein expressed in products containing Syngenta's corn event 5307(Agrisure Duracade®).

No cross-reactivity was detected with the following genetically modified traits/events in available corn seed:

GA21 (mEPSPS), Bt11 (Cry1Ab), mCry3A, or MIR162 (Vip3A).

Test Principle

The test system for eCry3.1Ab is a Double Antibody Sandwich (DAS) ELISA. Antibodies specific to eCry3.1Ab have been coated to the testwells of a microplate. An enzyme conjugate solution containing monoclonal antibodies specific to eCry3.1Ab protein conjugated to horseradish peroxidase has been included in this kit. The enzyme conjugate is added to the testwells followed by sample extracts. If eCry3.1Ab 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. The TMB substrate is added to the microplate. If the peroxidase conjugate is present a color will be produced signifying the presence of eCry3.1Ab. 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 has been evaluated in corn only. It is recommended for use with leaf samples, composite seed or grain, and single seed samples of corn.

Sample Extract Buffer: The eCry3.1Ab 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.

Corn Tissue	Tissue to Buffer Ratio
Leaf Sample	**1:20 (tissue weight in grams: buffer volume in mL)**
Single Seed	**1 seed:1 mL of 1X PBST**
Composite Seed (1 positive seed in 100 negative seeds)	**1:2 (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.

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 the provided incubation and development times. Timings for each sample type have been optimized to give the best results for both negative and positive samples.

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 lyophilized controls can be reconstituted with 2.0 mL of 1X PBST wash buffer per bottle.

Make control aliquots

After reconstituting the positive control, divide it 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

Leaves, seedlings, or seed must be ground and extracted in 1X PBST sample extraction buffer. Use the table below to determine the amount of buffer and extraction times required.

Tissue	Sample to Buffer Ratio	Example	Extraction Time
Single leaf	1:20 (weight:volume = g:mL)	0.15 g leaf: 3 mL buffer	10 minutes
Single seed	1 seed in 1 mL buffer	1 seed in 1 mL buffer	10 minutes
Seed sample (1 positive seed in 100 negative seeds)	1:2 (weight:volume = g:mL)	30 g:60 mL buffer	3 minutes

Single leaf -

1. 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. Use only one sample per bag and be sure to label each bag.
2. Insert the sample between the mesh linings near the bottom of the sample extraction bag.
3. Extract the sample by thoroughly macerating it with an Agdia tissue homogenizer or a blunt object such as a pen or permanent marker.
4. Add the appropriate volume of buffer and massage to mix the contents uniformly. An adequately extracted sample will result in a homogenous green or light brown colored solution.
5. Let the extract sit for the appropriate time listed in the table above.
6. Transfer 100 µL of the supernatant (upper liquid layer) to testwells of the ELISA plate.

Single seed -

1. 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.
2. Add the appropriate volume of buffer to crushed seed and massage to mix the contents uniformly.
3. Let the extract sit for the appropriate time listed in the table above.
4. Using wide-bore tip, transfer 100 µL of the supernatant (upper liquid layer) to testwells of the ELISA plate.

Seed sample -

1. For composite seed samples (up to 100 seeds) (presence of 1 positive seed in 100 negative seeds)
2. It is recommended to use a blender with a power rating of at least 450 watts in conjunction with "Mason" type Jars. The guidelines provided are optimized for Osterizer® blender with “Mason” type jars.
3. Put the seed sample in a dry “Mason” jar and assemble the blade attachment.
4. Grind the seed at high speed for 30 seconds or until all the seeds are ground to a fine powder. Dispense appropriate amount of buffer into jar, cap, and **shake vigorously** for at least 30 seconds.
5. Let the extract sit for the appropriate time listed in the table above.
6. Remove the cap, using wide-bore tip, transfer 100 µL of the supernatant (upper liquid layer) to testwells of the ELISA plate.

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.

Test Procedure

1. Prepare enzyme conjugate

The bottle of 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.

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 to determine the appropriate timing for each sample type:

Leaf Sample	Single-Seed Sample	Seed Sample
60 minutes	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 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 the TMB substrate solution into each well of the plate.

Let the plate incubate at room temperature according to the table below. Keep testwells away from strong light.

Leaf Sample	Single-Seed Sample	Seed Sample
20 minutes	20 minutes	30 minutes

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

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: Negatives should be rated ≤ 0.100 O.D. Positives for leaf and single seed should be rated at 0.500 O.D. or higher. Positives for grain should be rated at 0.300 O.D. or higher. Samples that obtain O.D. values greater than the buffer well value, but less than the positive O.D. threshold should be retested.

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 _____

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