

Bt-Cry1F ELISA Kit

Qualitative DAS ELISA for the detection of the Bt-Cry1F transgenic protein
Catalog number: PSP 10301

List of contents

| Lot number | Item | 96 wells | 288 wells | 480 wells | 4800 wells |
|------------|---|----------|-----------|-----------|------------|
| _____ | Antibody-coated 96-well microtiter plates | 1 | 3 | 5 | 50 solid |
| _____ | Peroxidase enzyme conjugate (1x, ready to use) | 11 ml | 33 ml | 55 ml | 550 ml |
| _____ | TMB substrate solution | 25 ml | 40 ml | 60 ml | 550 ml |
| _____ | Positive control | 1 | 1 | 1 | 5 |
| | The above items should be stored at 4°C | | | | |
| _____ | PBST wash buffer | 3 | 5 | 7 | 3 X 110 g |
| | The above items should be stored at room temperature. | | | | |

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 10301 - *Please specify leaf or seed control when ordering.*)
- 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
 - Balance 1-500 g
 - 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 (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 (4°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 detection of the HERCULEX® trait.

This test system can be used to test individual corn seed and corn leaf or detect as little as 1 transgenic Bt-Cry1F seed in 600 non-gmo corn seeds.

Test Principle

The test system for Bt-Cry1F is a direct DAS ELISA. Monoclonal antibodies specific to Bt-Cry1F are coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit containing monoclonal antibodies specific to Bt-Cry1F conjugated to a peroxidase enzyme. Enzyme conjugate is added to the testwells followed by sample extracts. If Bt-Cry1F 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-Cry1F. 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.

Samples: This test has been evaluated in corn only. The performance of this test with transgenic cotton samples has not been tested.

Sample Extraction Buffer: The Bt-Cry1F ELISA must be used with 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 (tissue weight in g: buffer volume in ml) dilution.

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

Color Development: Sample testwells from very concentrated positive samples may develop from a deep blue color to a green color after the addition of substrate. The green color will result in low O.D. values when read at the recommended 650 nm. Consider these samples as off scale positives. This rare occurrence is most likely to occur in single seed or single leaf samples.

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).

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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 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 | 5 g |
| Distilled water | 500 ml |

Prepare controls

Reconstitute lyophilized positive control and lyophilized negative control with 2.0 ml of prepared 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 μ l aliquots. If you will be using a control in two wells, prepare 220 μ l 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 (-20°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.

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Preparing Single Leaf and Seed Samples

Leaves, seedlings, or seeds must be ground and diluted in PBST sample extraction buffer. For best results, samples should be diluted in 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. Add the appropriate volume of buffer to an empty 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. Let the extract sit for 3 minutes before transferring sample to the testwells of the ELISA plate.

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.

Determine the average weight of the seed and add the appropriate volume of PBST buffer following a 1:2 to 1:5 (tissue weight in g: buffer volume in ml) seed to buffer ratio. Let the extract sit for 3 minutes before transferring to the testwells of the ELISA plate.

| Tissue | tissue to buffer ratio weight/volume - g/ml | Example | Extraction Time |
|-------------|---|---------------------------------------|-----------------|
| Single leaf | 1:20 | 0.15 g leaf: 3 ml buffer | 3 minutes |
| Single seed | 1:2 to 1:5 | Typically 1 seed in 1 ml of buffer | 3 minutes |

Preparing Grain Samples

For composite seed samples (up to 600 seeds), 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.

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.

Determine and record the average weight of 600 seeds. Put the seed sample in a dry "Mason" jar and assemble the blade attachment. A 1000 ml jar is recommended for 600 seeds. Grind the seed at high speed for about 60 seconds or until all the seeds are ground to a powder. Remove the jar from the blender and tap to collect all the powder. Shake the jar to mix and check for any unground seed.

Remove blade assembly and replace with threaded cap. Add the buffer at the specified ratio, close the lid and **vigorously shake** the bottle for 30 seconds. Let the sample settle for 3 minutes.

Use only the supernatant (liquid layer) when transferring the samples to testwells of the ELISA plate.

| Sample | grain to buffer ratio weight / volume - g/ml | Example | Extraction Time |
|--------|--|------------------------------|-----------------|
| Grain | 1:2 | 150 g seed: 300 ml buffer | 3 minutes |

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Test Procedure

1. Add enzyme conjugate

Dispense 100 µl of enzyme conjugate per well.

2. 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 PBST buffer into the buffer wells.

Mix the contents of the wells by gently swirling the plate on the bench-top.

3. Incubate plate

Set the plate inside the humid box and incubate at room temperature. Use the table to determine appropriate time for sample type.

| Single Leaf Sample | Single Seed Sample | Grain Sample |
|--------------------|--------------------|--------------|
| 60 minutes | 30 minutes | 2 hours |

4. 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.

5. 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.

6. 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.

| Single Leaf Sample | Single Seed Sample | Grain Sample |
|--------------------|--------------------|--------------|
| 10 minutes | 10 minutes | 20 minutes |

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7. Evaluate results

Examine the wells by eye, or measure 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 result. 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.

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 | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

