

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

List of contents

Lot number	Item	500 wells	1000 wells	5000 wells
_____	Capture antibody	0.275 ml	0.525 ml	2.525 ml
_____	Alkaline phosphatase enzyme conjugate	0.275 ml	0.525 ml	2.525 ml
_____	RUB3 enzyme conjugate diluent	11 ml	33 ml	55 ml
	The above items should be stored at 4° C.			
_____	General extract buffer 2 (GEB2)	55.7 g	2 X 55.7 g	3 x 204.4 g
_____	96-well uncoated microtiter plates	5	10	50
	The above items should be stored at room temperature.			

Materials required but not provided

- Distilled water
- Coating buffer, wash buffer and PNP substrate and PNP tablets - see page 6 for formulations or purchase an Agdia buffer pack (ACC 00113)
- Positive and Negative controls (Agdia catalog number LPC 14800 and LNC 14800)
- Paper towels
- Micropipette
- Micropipette tips
- Grinding device
 - Sample extract bag (catalog number ACC 00930) and tissue homogenizer (catalog number ACC 00900)
 - or
 - mortar and pestle
- Airtight container for incubations

Optional materials

- Postcoat 10 buffer – this buffer extends the life of coated plates by 8 weeks. This buffer is available from Agdia (catalog number ACC 00650).

Storing the kit

Store all kit components at the recommended temperature to assure their full shelf life. Do not store prepared 1X buffers from day to day.

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

Safety

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

Intended Use

This kit is intended for use with leaves displaying symptoms of infection and bacterial cultures, to determine the presence of *Acidovorax avenae* subsp. *citrulli* (*Aac*) in cucurbits. *Aac* is the causal agent of bacterial fruit blotch in cucurbits.

Test Principle

The test system for *Aac* is a direct DAS ELISA. Antibodies specific to *Aac* are coated to the testwells of a microplate. An enzyme conjugate solution has been included in this kit, containing monoclonal antibodies specific to *Aac* conjugated to alkaline phosphatase. Enzyme conjugate is added to the testwells followed by sample extracts. If *Aac* is present in the sample, it is bound by the antibody and captured on the microplate.

After a short incubation, the microplate is washed to remove any unbound enzyme conjugate and sample. PNP substrate is added to the microplate. If the alkaline phosphatase conjugate is present a color will be produced signifying the presence of *Aac*. The color reaction 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 leaves displaying symptoms of infection or bacterial cultures on solid media. This test should not be used with cultures from broth.

Sample Extract Buffer: The *Aac* ELISA must be used with GEB2 for optimal results. Do not use sample extract buffers used with other ELISA kits.

Sample Dilution: ELISA performance is very dependent on the proper sample 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.

Test Procedure: It is important to add the enzyme conjugate to the testwells before adding sample extracts.

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

Coat testwells of ELISA plate

1. Prepare humid box

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping the ELISA plates in a humid box during incubation will help prevent samples from evaporating.

2. Prepare coating antibody

Note: Coating antibody should be prepared in a container made of a material such as polyethylene or glass that does not readily bind coating antibody.

The coating antibody is provided in a concentrated solution and must be diluted with coating buffer before use. The recommended antibody to buffer ratio is given on the label.

Prepare the volume of coating buffer (formulation on page 6) needed for the test. You will need 100 μ l of coating antibody solution for each testwell you are using, therefore a 96 testwell plate requires 10 ml of coating buffer.

After the coating buffer is measured, add the appropriate volume of concentrated coating antibody to the coating buffer at the dilution given on the label. Use a new, sterile pipette tip to prevent contamination of the concentrated coating antibody.

Example: If the dilution given on the bottle of concentrated coating antibody is 1:200, and you are preparing 10 ml of coating antibody solution, you should mix 50 μ l of the concentrated coating antibody with 10 ml of coating buffer.

Mix the prepared coating antibody thoroughly and use immediately.

3. Coat plate

Pipette 100 μ l of the prepared coating antibody into each well.

4. Incubate plate

Incubate the plate in a humid box for 4 hours at room temperature or overnight in the refrigerator (4°C).

5. Wash plate

Empty the wells into a sink or waste container. Fill the testwells to overflowing with 1X PBST, then quickly empty them again. Repeat 2 more times.

Hold the plate upside down and tap firmly on a folded paper towel to remove excess liquid.

Note: Use freshly coated plates immediately. If you would like to store the plates for future use it is necessary to apply Postcoat 10, which is available separately from Agdia. (Catalog number ACC 00650).

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

Prepare Samples

When possible, select samples showing symptoms. Leaf tissue is often used in ELISA testing. Stem, petiole, and bacterial culture can also be tested. It is necessary to completely grind samples in GEB2 sample extraction buffer (directions on page 6).

Plant Tissue Samples

Grind plant tissue in GEB2 sample extraction buffer at a ratio of 1:10 (g/ml). Leaves can be ground in Agdia mesh sample bags or with a mortar and pestle. If using a mortar and pestle be sure to clean it between samples.

Bacterial Culture Samples

Bacterial culture samples can also be tested. Use a toothpick to remove a colony of bacteria from a culture plate. Stir the toothpick into a tube containing 300 μ l of GEB2 sample extraction buffer. Do not use Aac ELISA with cell culture broth.

Use only the supernatant (liquid layer) when adding sample extracts to testwells.

Prepare controls

Reconstitute the bottle of lyophilized positive control or negative control with 2.0 ml GEB2 extract buffer.

Make control aliquots

After reconstituting 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.

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

Test Procedure

- 1. Add enzyme conjugate**

Dispense 100 μ l of enzyme conjugate per testwell and then wait 5 minutes before performing the next step.
- 2. Dispense samples / controls**

Following your loading diagram, dispense 100 μ l of each prepared sample into the appropriate testwells of the ELISA plate. Add 100 μ l 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.
- 3. Incubate plate**

Set the plate inside the humid box and incubate 2 hours at room temperature or overnight in the refrigerator (4°C).
- 4. 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 another testwell.

Fill all the testwells to overflowing with 1X PBST, and quickly empty. **Repeat this PBST wash step 6 to 8 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 excess liquid.

Note: If using an automatic plate washer please be sure that the machine is at the appropriate settings for washing flat bottom plates.
- 5. Add substrate solution**

Add 100 μ l of PNP substrate solution to each well. Set plate aside and wait for color development.
- 6. Measure color**

Color will develop over the course of 60 minutes. Measure the optical density of the testwells on a plate reader at 405 nm or visually. Wells in which a yellow color develops indicate positive results for *Aac*. Wells remaining colorless indicate negative results. If either control well does not show the appropriate color, please repeat the test procedure. If problems persist contact Agdia for further assistance.

Technical service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (1-574-264-2014 or 1-800-622-4342) or by email (info@agdia.com).

Aac Reagent Set

ELISA for the detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: SRA 14800

Buffer formulations

Coating buffer (1X)

Dissolve in distilled water to 1000 ml:

Sodium carbonate (anhydrous)	1.59 g
Sodium bicarbonate	2.93 g
Sodium azide	0.2 g

Adjust pH to 9.6. Store at 4° C.

General extract buffer 2 (GEB2)

GEB2 is used to dilute and extract samples. Refer to the sample preparation section for appropriate sample to buffer ratios. GEB2 should be prepared by dissolving GEB2 powder in distilled water.

Buffer powder	27.9 g
Distilled water	500 ml

Stir for 30 minutes. Store at 4° C.

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

PNP substrate (1X)

Dissolve in 800 ml distilled water:

Magnesium chloride hexahydrate	0.1 g
Sodium azide	0.2 g
Diethanolamine	97.0 ml

Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C. About 15 minutes before the substrate step, measure the volume of 1X PNP buffer needed. Then, without touching the tablets (5 mg each), add one PNP tablet for every 5 ml of buffer.

Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

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												

