PathoScreen® Kit

ACP ELISA, alkaline phosphatase label

List of contents

Lot number	Item	96 wells	288 wells	480 wells
_____	Detection antibody	0.150 mL	0.350 mL	0.550 mL
_____	Alkaline phosphatase enzyme conjugate	0.150 mL	0.350 mL	0.550 mL
_____	Indirect sample extraction buffer, 10X concentrate	60 mL	120 mL	120 mL
_____	ECI buffer, 5X concentrate	15 mL	30 mL	30 mL
_____	PNP substrate tablets, 5 mg each	12	12	25
_____	PNP substrate buffer, 5X concentrate	12 mL	12 mL	25 mL
_____	Positive control (if available)	1	1	1
	The above items should be stored at 2 - 8 °C			
_____	96 well microtiter plate, strip	1	3	5
_____	PBST wash buffer, 20X concentrate, 50 mL	3	5	7
	The above items should be stored at room temperature (18 - 30 °C).			

Materials required, but not provided

- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Lyophilized negative control can be purchased from Agdia
- Sample grinding device such as:
    - Agdia sample mesh bag (ACC 00930)
    - Agdia tissue homogenizer (ACC 00900)
    - Mortar and pestle
- Airtight container for incubations

Limitations

Expiration: This test should be used within 1 year of purchase.

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.

Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use. Buffer formulations on page 6 are for reference only.

Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Sample Buffer: Indirect sample extraction buffer must be used to achieve optimal results. This buffer promotes binding of the virus to the microtiter plate.

Precautions

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.

Preparing for the test

Familiarize yourself with the kit components. Check that all components are present in the kit.

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).

Prepare buffers

Prepare only the amount of 1X buffers needed for the day.

PBST wash buffer

Prepare PBST wash buffer by diluting one 20X pouch of PBST wash buffer with 950 mL of distilled water.

Indirect sample extraction buffer

Indirect sample extraction buffer is used to dilute and extract samples. It is used at a sample to buffer ratio of 1:100 (1:200 for AV2) (tissue weight in g: buffer volume in mL).

To prepare 10 mL of working indirect sample extraction buffer, mix 1 mL of indirect sample extraction buffer concentrate with 9 mL of distilled water.

ECI buffer

ECI buffer is used to dilute the concentrated enzyme conjugate. The volume of 1X ECI buffer required depends on the number of testwells used. You will need 100 µL of prepared ECI buffer for each testwell you are using. To estimate the volume needed, prepare 1 mL for each 8 well strip used. A full plate will require about 10 mL.

To prepare 10 mL of working ECI buffer, mix 2 mL of 5X ECI buffer concentrate with 8 mL of distilled water.

PNP substrate buffer

PNP substrate buffer is used to dilute PNP substrate tablets. The volume of 1X PNP buffer required depends on the number of testwells used. You will need 100 µL of prepared PNP substrate buffer for each testwell you are using. To estimate the volume needed, prepare 1 mL for each 8 well strip used. A full plate will require about 10 mL.

To prepare 10 mL of working PNP substrate buffer, mix 2 mL of 5X PNP buffer concentrate with 8 mL of distilled water.

Note: PNP substrate tablets will not be added at this time. Tablets will be added prior to completion of the enzyme conjugate incubation.

Prepare controls

Reconstitute the bottle of lyophilized positive control and lyophilized negative control with 2.0 mL indirect 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 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 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

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 and positive control well on each plate each time you run the test.

Grind and dilute samples

When possible, select samples showing symptoms. Leaf tissue is often used in ELISA testing. Stem, seed, and other tissue can also be tested. In some cases, composites of up to ten leaves per testwell can be used to make testing more economical. However, too many plant samples per well can reduce the sensitivity of the test.

Use Agdia's indirect sample extraction buffer to grind and dilute samples.

Grind plant tissue in indirect sample extraction buffer at a 1:100 ratio (tissue weight in g: buffer volume in mL). You will need 100 µL of diluted sample extraction per testwell, plus an additional amount to assure easy dispensing. You can use Agdia's sample mesh bags (ACC 00930), Agdia's tissue homogenizer (ACC 00900), a mortar and pestle, or other grinding devices to grind samples. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.

Exceptions

If testing for *Asparagus virus* 2 using Agdia test system number 71000, grind tissue in indirect sample extraction buffer at a 1:200 ratio (tissue weight in g: buffer volume in mL).

Test Procedure

This is an antigen coated plate (ACP) ELISA in which the sample is coated directly to an empty microtiter plate.

1. Dispense samples

Following your loading diagram, dispense 100 µL of prepared sample into sample wells. Dispense 100 µL of positive control into positive control wells, and dispense 100 µL of indirect sample extraction buffer into buffer wells. If you will be using a negative control, dispense 100 µL into negative wells.

2. Incubate plate

Set the plate inside the humid box and incubate for 1 hour at room temperature.

3. Prepare antibody

Note: All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polystyrene.

Note: Always prepare antibody within 10 minutes before use.

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

Prepare the volume of ECI buffer needed for the test. You will need 100 µL of ECI buffer for each test well you are using. A full plate will require about 10 mL. Then, add the appropriate volume of concentrated detection antibody to the ECI buffer at the dilution on the label.

Example: If the dilution given on the bottle of concentrated detection antibody is 1:100, and you are preparing 10 mL of detection antibody solution, you should mix 10 mL of ECI buffer with 100 µL of the concentrated detection antibody. Mix the prepared detection antibody solution thoroughly and use immediately.

4. 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.

Inspect the testwells. All wells should be free of plant tissue. If tissue is present repeat the wash step and tap firmly on a paper towel.

5. Add antibody

Dispense 100 µL of prepared antibody per well.

6. Incubate plate

Incubate the plate in the humid box for 2 hours at room temperature.

7. Prepare enzyme conjugate

Note: Always prepare enzyme conjugate within 10 minutes before use.

The bottle of alkaline phosphatase enzyme conjugate is supplied as a concentrate and must be diluted with ECI buffer before use. The recommended conjugate to buffer ratio is given on the label. Dispense the appropriate volume of prepared ECI buffer into a dedicated container. You will need 100 µL of buffer for each testwell you are using. Then, add the alkaline phosphatase enzyme conjugate according to the dilution given on the labels.

Example: If the dilution given on bottle of concentrated alkaline phosphatase enzyme conjugate is 1:100, and you are preparing 10 mL of enzyme conjugate solution, you should first dispense 10 mL ECI buffer. Then, add 100 µL of the concentrated enzyme conjugate to the ECI buffer.

After adding the enzyme conjugate, mix thoroughly. It is important to mix the enzyme conjugate solution well.

8. Wash plate	As before, wash the plate 8 times with 1X PBST. Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.
9. Add enzyme conjugate	Dispense 100 µL of prepared enzyme conjugate per well.
10. Incubate plate	Incubate the plate in the humid box for 1 hour at room temperature.
11. Prepare PNP solution	Each PNP tablet (ACC 00404) will make 5 mL of PNP solution, at a concentration of 1 mg/mL, about enough for five 8-well strips. About 15 minutes before the end of the above incubation step, measure 5 mL of room temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the 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.**
12. Wash plate	As before, wash the plate 8 times with 1X PBST. Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.
13. Add PNP solution	Dispense 100 µL of PNP substrate into each testwell.
14. Incubate plate	Incubate the plate for 60 minutes. Plates should be protected from direct or intense light.
15. Evaluate results	Examine the wells by eye, or measure on a plate reader at 405 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 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. Results may be interpreted after more than 60 minutes of incubation as long as negative wells remain virtually clear.

Buffer Formulations

Indirect Sample Extraction 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
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0 g

Adjust pH to 9.6. Store at 2 - 8 °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

ECI Buffer (1X)

Add to 1000 mL 1X PBST:

Bovine serum albumin (BSA)	2.0 g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0 g
Sodium azide	0.2 g

Adjust pH to 7.4. Store at 2 - 8 °C.

PNP Buffer (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 2 - 8 °C.

Date _____ Test _____

Test performed by _____

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