

Reagent Set

DAS ELISA, Alkaline phosphatase label

List of contents

Lot number	Item	96 wells	500 wells	1000 wells	5000 wells
_____	Capture antibody	0.150 ml	0.275 ml	0.525 ml	2.525 ml
_____	Alkaline phosphatase enzyme conjugate	0.150 ml	0.275 ml	0.525 ml	2.525 ml
	<i>The above items should be stored at 4° C.</i>				
_____	96-well microtiter plates, strip or solid	1 strip	5 solid	10 solid	50 solid
	<i>The above items can be stored at room temperature.</i>				

Materials required, but not provided

- Carbonate Coating Buffer (formulation on page 5)
- PBST Wash Buffer (formulation on page 5)
- ECI Buffer (formulation on page 5)
- PNP Substrate Buffer (formulation on page 5)
- PNP Substrate Tablets (ACC 00404)
- General Extract Buffer (formulation on page 5)
- Buffer packs containing the above required items can be purchased from Agdia (ACC 00111)
- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Sample grinding device such as:
 - Agdia sample mesh bag (ACC 00930)
 - Agdia tissue homogenizer (ACC 00900)
 - Mortar and pestle
- Airtight container for incubations
- **When testing blueberry leaves or other acidic hosts, the recommended extraction buffer is Agdia's General extract buffer 3 (GEB3) (ACC 00360).**

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.

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.

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.

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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Coating testwells of ELISA plate

1. Prepare humid box 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.

2. Prepare capture 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: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on capture antibody bottle for appropriate dilution ratio.

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

Prepare the volume of carbonate coating buffer needed for the test. You will need 100 µl of carbonate coating buffer for each test well you are using. A full plate will require about 10 ml. Then, add the appropriate volume of concentrated capture antibody to the carbonate coating buffer at the dilution on the label.

Example 1: If the dilution given on the bottle of concentrated capture antibody is 1:200, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 50 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

Example 2: If the dilution given on the bottle of concentrated capture antibody is 1:100, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 100 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

3. Coat plate Pipette 100 µl of the prepared capture 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). Do not store coated plates longer than 24 hours. If long term storage is desired, contact Agdia about postcoat buffers.

5. Wash plate Empty the wells into a sink or waste container. Fill the testwells completely with 1X PBST, and 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.

Grind and dilute samples

When possible, select samples showing symptoms. Leaf tissue is primarily used in ELISA testing however; stem, seed, and other tissue can also be tested. Please note that various tissues even from the same plant may have different pathogen concentrations. In some cases, composites of up to ten leaves per test well can be used to make testing more economical, but it is important to not composite too many plant samples per well as sensitivity of the test can be adversely affected. **For test specifics and limitations please visit our website.**

Use Agdia's general extract buffer (GEB) to grind and dilute samples.

Grind plant tissue in sample extraction buffer at a 1:10 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

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mortar and pestle, wash and rinse it thoroughly between samples.

Test Procedure

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 sample extraction buffer into buffer wells.

2. Incubate plate

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

3. 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.

Note: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on enzyme conjugate bottle for appropriate dilution ratio.

Example 1: If the dilution given on bottle of concentrated alkaline phosphatase enzyme conjugate is 1:200, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml of ECI buffer. Then, add 50 µl of the concentrated enzyme conjugate to the ECI buffer.

Example 2: 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.

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 enzyme conjugate

Dispense 100 µl of prepared enzyme conjugate per well.

6. Incubate plate

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

7. 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.

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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 PNP substrate
Dispense 100 µl of PNP substrate into each testwell.
10. Incubate plate
Incubate the plate for 60 minutes. Plates should be protected from direct or intense light.
11. 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.

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Buffer Formulations

Carbonate 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.

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 4° 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 4° C.

General Extract Buffer (GEB 1X)

Dissolve in 1000 ml of 1X PBST:

Sodium sulfite (anhydrous)	1.3 g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0 g
Sodium azide	0.2 g
Powdered egg (chicken) albumin, Grade II	2.0 g
Tween-20	20.0 g

Adjust pH to 7.4. Store at 4°C.

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												