

## PathogenID TEST Kit

### DAS ELISA, alkaline phosphatase conjugate

Lot number	Item	96 Wells	192 Wells	384 Wells	Storage
	96-well plates coated with antibody	1	2	4	2-6°C
	Detecting conjugate, alkaline phosphatase, 1x	12 ml	23 ml	45 ml	2-6°C
	BluePhos substrate, Part A and Part B, 1x	A: 6ml B: 6ml	A: 11ml B: 11ml	A: 22ml B: 22ml	2-6°C
	ELISA control, positive, 1.8 ml/bottle	1	1	2	2-6°C
	ELISA control, negative, 1.8 ml/bottle	1	1	2	2-6°C
	PBST Washing buffer, powder	14.4 g	28.7 g	57.3 g	RT*
	Tween-20, for PBST and sample buffer	4.0 g	7.0 g	14.0 g	RT
	SB1 General Sample buffer, powder**	5.8 g	11.6 g	23.2 g	RT
	SB4 grape sample buffer, powder**	24.7 g	49.4 g	98.7 g	RT
	SB5 blueberry sample buffer, powder**	8.6 g	17.3 g	34.5 g	RT

\*RT = Room Temperature

\*\*Only one of the three buffers is included

#### The following materials are not included, but required:

- Pipette and pipette tips
- Distilled water or other purified water
- Humid incubation container
- Glass wares, plastic wares, paper towels or other lab supplies
- 2.5% EDTA stopping solution (optional)

#### Safety and Storage

Always wash hands thoroughly after using this product. Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of reagent components.

All reagent components should be stored at the recommended temperature to assure their full shelf life. The kit should be used within six months of purchase.

Please contact AC Diagnostics, Inc. if you have any questions about safety and storage of this product.

#### Preparing For the Test

Make sure all laboratory equipments and facilities required are ready for the test. Prepare a humid box for incubation steps

#### Kit Components

Check all the components are present in the package of PathogenID Kit by referring to the Content List. Familiarize yourself with the listed components before starting the test.

#### Prepare Buffers from Powders

To prepare the 1x buffers, dissolve the buffer powder into D.H<sub>2</sub>O and add tween-20 at the ratios on the table below. For sample buffer, mix the powder with small amount of D.H<sub>2</sub>O into a paste (no clumps) before adding more D.H<sub>2</sub>O. Stir for 10-30 minutes for dissolving completely and make up to final volume.

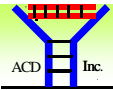
	1x Sample buffer			1x PBST buffer		
Buffer Powder	5.8 g	11.6 g	23.2 g	14.4 g	28.7 g	57.3 g
Tween-20	2.5 g	5.0 g	10.0 g	0.8 g	1.5 g	3.0 g
Final Volume	250 ml	500 ml	1000 ml	1500 ml	3000 ml	6000 ml

Prepared 1x working solution can be stored at refrigerator (2-6°C) for up to 3 months. If you have any questions about preparing and using the buffers, please contact AC Diagnostics.

#### Prepare Controls

Add 1.8 ml of sample extraction buffer into the bottles of lyophilized positive and negative controls and mix by gently inverting the bottles until fully dissolved.

The prepared control can be used immediately, or divided into aliquots and stored frozen (-10 to -40°C). Each aliquot should be sufficient for at least one use. For example, if you will use this control in one well each time you run the test, prepare 120 µl aliquots. Prepare 220 µl aliquots if you will use the control in two wells.



Control aliquots must be kept frozen until just before use. Do not refreeze controls once they have been thawed. Using the Control at the time you run the test, remove one control from storage and allow it to thaw. Add 100 µl of the prepared control to the appropriate control well.

### Prepare Test Wells

If only part of the 96-well plate to be used, remove the unused strips and seal them in the foil pouch with the desiccant. Mark the strips in case a strip becomes separate from the frame.

Make a copy of the attached recording sheet and create a loading diagram by recording the locations of your samples, controls, and other reagents needed.

### Prepare Samples

Select symptomatic and/or infective tissues for the test. Leaf tissue is often used in ELISA testing. However, plant tissues such as stem, sprout, seed, tuber, root and others can also be tested.

Single sample is suggested to be used in each test well. In some cases, composites of up to ten leaves per test well can be used to make testing more economical. However, too many plant samples per well can reduce the sensitivity of the test.

AC Diagnostics' SBI buffer can be used as extraction buffer for most of plant samples. However, other buffers are also recommended for grape, blueberry or some plant species.

Grind sample with a mortar and pestle, or other grinding devices. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.

If you extract plant sap, dilute the sap into sample extraction buffer at a ratio of 1:10 (sap volume: buffer volume). Or you can grind plant tissue in extraction buffer at a 1:10 ratio (tissue weight: Extraction Buffer volume).

If you have any questions about sampling, sample preparation, or the appropriate extraction buffer for your samples, please contact AC Diagnostics, Inc.

## ELISA Test Procedure

### Sample Dispensing

Following your loading diagram on your recording sheet, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and 100 µl of negative control into negative control wells.

### Plate Incubation

Put the plate inside the humid box and incubate for 2.5 hours at room temperature (21-24 °C) or overnight in the refrigerator (4° C).

### Washing Plate

Wash the plate when the incubation is complete. While squeezing the long side of the frame to hold the strips in place, use a quick flipping motion to empty the wells into a sink or waste container without mixing the contents.

Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 6 to 8 times. To remove drops of PBST from the wells after washing, hold the frame upside down and tap firmly on a folded paper towel.

### Enzyme Conjugate Incubation

Enzyme conjugate is 1x solution and ready to use. Do not pipette from the conjugate bottle into test wells directly. Measure out the volume needed for the test before dispensing.

Dispense 100 µl of the enzyme conjugate per well. Incubate the plate in the humid box for 2.5 hours at room temperature (21-24 °C).

### Washing plate

Wash the plate 6 to 8 times with PBST as above. Wash once with distilled water and tap firmly on the paper tower to remove any bubbles in the wells.

### Incubation with Substrate

BluePhos substrate includes two parts of 1x solutions and is ready to use. Mix equal volumes of Part A and Part B in a container immediately before use. Mixed substrate exhibits a clear yellow color. Use within 30 minutes of mixing.

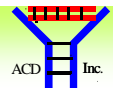
Dispense 100 µl of prepared substrate solution per well. Incubate the plate for 30-60 minutes at room temperature (21-24 °C)

To stop reaction, add 50 µl of 2.5% EDTA solution to each well. This step is optional. The plate can be interpreted visually or with a plate reader without adding the stop solution.

### Evaluating Results

Test results can be examined by eye, or measured on a plate reader at 595-650 nm.

Development of blue color in test wells indicates positive results. Wells in which there is no significant color development indicate negative results. Test results are



valid only if positive control wells give a positive result and negative control wells remain clear.

Results may be interpreted before 30 minutes or after more than 60 minutes of incubation as long as negative control wells remain virtually clear.

## Buffer Formulations for your Reference

### Antibody Coating Buffer

Sodium carbonate (anhydrous)	1.60 g
Sodium bicarbonate	2.92 g
Sodium azide	0.2 g

Dissolve in distilled water to 1000 ml. Adjust pH to 9.6. Store at 4° C.

### PBST Wash Buffer

Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Sodium chloride	8.0 g
Potassium chloride	0.2 g
Tween-20	0.5 g

Dissolve in distilled water to 1000 ml. Adjust pH to 7.3.

### ECB1 Enzyme Conjugate Buffer

Bovine serum albumin (BSA)	2.0 g
Polyvinylpyrrolidone (PVP) MW 24-40,000	10.0 g
Sodium azide	0.2 g

Dissolve in 1000 ml 1X PBST. Adjust pH to 7.3. Store at 4° C.

### SB1 Sample Buffer

Powdered egg (chicken) albumin, Grade II	2.0 g
Polyvinylpyrrolidone (PVP) MW 24-40,000	10.0 g
Sodium sulfite (anhydrous)	1.3 g
Sodium azide	0.2 g
Tween-20	10.0 g

Dissolve in 1000 ml 1X PBST. Adjust pH to 7.3. Store at 4° C.

### SB4 Grape Sample Buffer

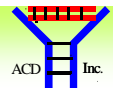
Tris base (0.5 M)	60.5 g
Sodium chloride (0.8%)	8.0 g
Polyvinylpyrrolidone (PVP), MW 24-40,000 (2%)	20.0 g
Polyethylene glycol (1%)	10.0 g
Sodium azide ((0.02%))	0.2 g
Tween-20 (0.05%)	0.5 ml

Dissolve in 900 ml distilled water, adjust pH to 8.2 with Hydrochloric acid, and make to 1000 ml with distilled water

### SB5 Blueberry sample extraction buffer

Sodium phosphate, dibasic (Anhydrous) (0.01 M)	10.4 g
Potassium phosphate, monobasic (0.01M)	0.9 g

Dissolve in General Sample Extraction Buffer to 1000 ml



### RECORDING SHEET FOR ELISA

TEST: \_\_\_\_\_ DATE: \_\_\_\_\_ BY: \_\_\_\_\_

TIMING: Coating \_\_\_\_\_ Sample \_\_\_\_\_ EC \_\_\_\_\_ Substrate: \_\_\_\_\_

KEY POINTS: \_\_\_\_\_

Coating Antibody: \_\_\_\_\_ ul, Coating Buffer: \_\_\_\_\_ ml,

Enzyme Conjugate: \_\_\_\_\_ ul, ECB1 \_\_\_\_\_ ml

PNP Substrate: \_\_\_\_\_ ml

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

### RESULTS/CONCLUSIONS:

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_