

PathogenID TEST Kit

Indirect ELISA, alkaline phosphatase conjugate

| Lot number | Item | 96 Wells | 192 Wells | 384 Wells | Storage |
|------------|--|------------------|--------------------|--------------------|---------|
| | SB3 Indirect sample buffer, 10X | 25 ml | 50 ml | 100 ml | 2-6°C |
| | Detecting conjugate, alkaline phosphatase, Bottle A & Bottle B, 1x | A: 6ml B: 6ml | A: 12ml B: 12ml | A: 23ml B: 23ml | 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 |
| | | | | | |
| | 96-well ELISA plates, empty | 1 | 2 | 4 | RT* |
| | PBST Washing buffer, powder | 14.4 g | 28.7 g | 57.3 g | RT |
| | Tween-20, for PBST and sample buffer | 1.0 g | 2.0 g | 4.0 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 1x Buffers

For 1x sample buffer, dilute the concentrate buffer at 1:10 with D.H₂O. To prepare the 1x PBST buffer, dissolve the buffer powder into D.H₂O and add tween-20. Refer to the ratios on the table below for preparing the buffers. Stir the prepared buffers for 10-30 minutes for dissolving completely.

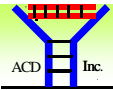
| | 1x Sample buffer | | | 1x PBST buffer | | |
|---------------|------------------|--------|---------|----------------|---------|---------|
| Buffer Powder | 25 ml | 50 ml | 100 ml | 14.4 g | 28.7 g | 57.3 g |
| Tween-20 | 0 | 0 | 0 | 0.8 g | 1.5 g | 3.0 g |
| Final Volume | 250 ml | 500 ml | 1000 ml | 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' SB3 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 1:100 (sap volume: buffer volume). Or you can grind plant tissue in extraction buffer at a 1:100 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 includes Bottle A and Bottle B, which are 1x solutions and ready to use. Do not pipette from the bottle into test wells directly. Mix equal volumes of Bottle A and Bottle B in a container immediately before use. Make a mixture in the volume needed for the test.

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 towel 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.

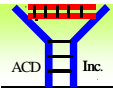
Dispense 100 µl of prepared substrate solution per well. Incubate the plate for 30-60 minutes at room temperature (21-24 °C)

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 posi-



tive 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

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

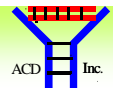
| | |
|---|--------|
| Bovine serum albumin (BSA) | 2.0 g |
| Polyvinylpyrrolidone (PVP) MW 24-40,000 | 10.0 g |
| Sodium azide | 0.2 g |

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

Indirect sample buffer: For Indirect-ELISA only

| | |
|---|--------|
| Sodium carbonate | 1.59 g |
| Sodium bicarbonate | 2.93 g |
| Sodium azide (0.2%) | 0.2 g |
| Polyvinylpyrrolidone (PVP), MW 24-40,000 (2%) | 20.0 g |

Dissolve in distilled water to 1000 ml. Adjust pH to 9.6



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. _____