# ELISA Reagents

## Indirect ELISA, alkaline phosphatase conjugate

### Content List

| Lot Number | Item | 500 wells | 1000 wells | 5000 wells | Storage |
|---|---|---|---|---|---|
| | Detecting conjugate, alkaline phosphatase (Bottle A and B) | 0.25 ml (A) 0.25 ml (B) | 0.50 ml (A) 0.50 ml (B) | 2x1.25 ml (A) 2x1.25 ml (B) | 4 ºC |
| | 96-well ELISA plates | 5 | 10 | 50 | Room temperature |
| | Instruction | 1 | 1 | 1 | |

### 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. Do not store prepared working solution from day to day.**

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

### Preparing For The Test

**Check all the components in the package of ELISA Reagents.**

**Prepare all of buffer solutions according to the attached buffer formulations.**

**Make sure all laboratory equipments and facilities required for the test are ready.**

**Prepare a humid box for incubation steps.**

**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.**

### Preparing 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. Remove any soil from the tissue to be tested since contamination with soil can interfere with ELISA tests.**

**Most plant sample should use ACD's SB3 buffer as the extraction buffer. Grind sample with a mortar and pestle, or other grinding devices. If you are using a mortar and pestle, wash and rinse them thoroughly between samples.**

**If you extract plant sap, dilute the sap into SB3 sample extraction buffer at a ratio of 1:100 (sap volume: buffer volume). Or you can grind plant tissue in SB3 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 ACD, Inc.**

### Sample Dispensing and Incubation

**Approximately 100 µl of diluted sample extract is needed per test well. Always have an additional amount to assure easy dispensing. A convenient way to prepare this diluted sample is to measure 10 µl of undiluted sap into a small test tube, then add 1 ml of extraction buffer.**

**Dispense the prepared samples into wells of ELISA plate following your loading diagram on your recording sheet. Add 100 µl of positive control into positive control well and 100 µl of negative control or extraction buffer into negative control well.**

**Put the plate inside the humid box and incubate for 2.0 hours at room temperature (21-24 °C).**

### Preparing Enzyme Conjugate

**Always make enzyme conjugate solution within 10 minutes before use. Prepare the enzyme conjugate, using ACD's ECB1 buffer and a clean container.**

**The volume of ECB1 buffer required depends on the number of test wells used; 100 µl is needed per test well. To estimate the volume needed, prepare 1 ml for each 8-well strip used, or 10 ml for each 96-well plate.**

**The volume of enzyme conjugate required for each test is calculated based on the volume of ECB1 buffer used and on the dilutions given on the bottles. To prevent contamination, use a new and sterile pipette tip each pipetting of each bottle.**

**Dispense appropriate volume of ECB1 buffer into a clean container, then add enzyme conjugate from bottle A and from bottle B according to the dilutions given on the label.**

**For example, if the dilutions given on bottles A and B are both 1:200 and you are preparing 2 ml of enzyme conjugate, you should first dispense 2 ml of ECB1**

buffer. Then add 10 µl from bottle A and 10 µl from bottle B to the ECB1 buffer.

After adding the conjugates from bottles A and B, mix the conjugate solution thoroughly. If you prepare the conjugate in a test tube, invert it several times. If you prepare the conjugate in a beaker, stir the conjugate solution with a glass rod. It is important to mix the enzyme conjugate well for a consistent test result.

Prepare enzyme conjugate just before use. Keep the prepared enzyme conjugate at a safe place and use it after washing the plate.

## Plate Washing

Wash the plate when the incubation is complete. 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

Dispense 100 µl of prepared enzyme conjugate per well for all test wells.

Incubate the plate in the humid box for 2.0 hours at room temperature (21-24 °C).

## Preparing Substrate Solution

The concentration of PNP in substrate is 1 mg/ml. Each PNP tablet will make 5 ml of PNP solution, which is enough for five 8-well strips.

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

Prepare PNP substrate 10-15 minutes before the end of the above incubation step. Measure 5 ml of PNP buffer for each tablet, then add the PNP tablets to the buffer. Mix by vortexing or stirring to let the PNP tablet fully dissolve in the buffer.

## Plate Washing

Wash the plate 6 to 8 times with PBST as above. Then fill the wells with PBST and keep soaking the wells for 2-3 minutes. Empty the wells and tap on a folded paper towel.

## Incubation With Substrate

Dispense 100 µl of PNP substrate solution per well.

Incubate the plate for 30 to 60 minutes in a humid box at room temperature (21-24 °C).

To stop the reaction, add 50 µl of 3M sodium hydroxide 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 405 nm.

Development of yellow color in test wells indicate positive results. Wells in which there is no significant color development indicates 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 after more than 60 minutes of incubation as long as negative control wells remain virtually clear.

## Buffer Formulations

### SEB3 Buffer

| | |
|---|---|
| Sodium carbonate (anhydrous) | 1.60 g |
| Sodium bicarbonate | 2.92 g |
| Polyvinylpyrrolidone (PVP) MW 24-40,000 | 10.0 g |
| Sodium azide | 0.2 g |

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

### PBST 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 and make to 1000 ml. Adjust pH to 7.3.

### ECB1 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 of 1X PBST. Adjust pH to 7.3. Store at 4° C.

### PNP Buffer

| | |
|---|---|
| Diethanolamine | 97.0 ml |
| Magnesium chloride | 0.1 g |
| Sodium azide | 0.2 g |

Dissolve in 800 ml distilled water. Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C.

# RECORDING SHEET OF ELISA

**TEST: ______________________ DATE: ____________ BY: ____________**

**TIMING: Coating ________ Sample ________ EC ________ Substrate: ________**

**KEY POINTS: ______________________________________________**

**Coating Sample: ____________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.______________________________________________**