ELISA Reagents
TAS ELISA, alkaline phosphatase conjugate

Content List

Lot Number	Item	500 wells	1000 wells	5000 wells	Storage
	Coating antibody	0.25 ml	0.50 ml	2x1.25 ml	4 °C
	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 °C
	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.

Coating Plate With Antibody

Lay out all items that will be required for the plate coating step before you begin. Prepare coating antibody in a container made of glass, polyethylene or a material that will not readily bind coating antibody. We suggest the plate be coated immediately after preparing the coating antibody. Some coating antibody can be lost if too much time elapses between diluting the coating antibody and coating the plate.

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

Dilute the concentrated coating antibody into the coating buffer at the dilution given on the label. Mix well. Always prepare coating antibody immediately before use.

Pipette 100 µl of coating antibody into each well.

Incubate the plate in a humid box overnight in a refrigerator (4 °C) or for 4 hours at room temperature (21-24 °C).

Preparing Samples

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

We suggest that use each test well for only a single sample. 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.

ACD's SB1 buffer is recommended as extraction buffer for most of the plant samples. However, other buffers can also be used for 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 them 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 ACD, Inc.

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.

. Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 4 to 6 times.

To remove drops of PBST from the wells after washing, hold the frame upside down and tap firmly on a folded paper towel.

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 100 µl of undiluted sap into a small test tube, then add 1 ml of extraction buffer.

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

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).

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. Use a new, sterile pipette tip and change the tip for each pipetting to prevent contamination.

First dispense appropriate volume of ECB1 buffer into a clean container, then add enzyme conjugate from bottle A and 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 2.5 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 instructed above.

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 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 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 after more than 60 minutes of incubation as long as negative control wells remain virtually clear.

Buffer Formulations

Coating Buffer

Sodium carbonate (anhydrous)	1.60 g
Sodium bicarbonate	2.92 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.

SB1 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 of 1X PBST. Adjust pH to 7.3. Store at 4° C.

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 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. _____