primediagnostics

Plant Research International

Double Antibody Sandwich (DAS) ELISA for detection of plant virus

Buffers and chemicals:

Coating buffer:	1.59 gr Na_2CO_3 2.94 gr $NaHCO_3$ 0.5 gr NaN_3 pH 9.6 Add distilled demineralized water to 1000 ml total volume
PBS 0.01 M:	8 gr NaCl 1 gr KH_2PO_4 14.5 gr Na_2HPO_4 x 12 H_2O 0.5 gr NaN_3 800 ml distilled demineralized water pH 7.4 with NaOH Add distilled demineralized water to 1000 ml total volume
Washing buffer: (PBST)	0.1 % Tween-20 in 0.01 M PBS
Extraction buffer: (SEB)	0.1 % Tween-20 2 % polyvinylpyrrolidone (PVP-25) (0.2 % ovalbumine, grade VI) In 0.01 M PBS
Substrate buffer:	97 ml diethanolamine 600 ml distilled demineralized water 0.5 gr NaN_3 pH 9.8 with HCl Add distilled demineralized water to 1000 ml total volume
Substrate:	5 mg paranitrophenylphosphate (pNPP) tablets, Sigma104-105 3 tablets in 20 ml substrate buffer
Coating antibody:	Purified polyclonal virus-specific antibody (IgG) raised in rabbit. Concentration is 1 mg IgG/ml
AP Conjugate:	Alkaline Phosphatase conjugated polyclonal virus-specific antibody (IgG), raised in rabbit. Concentration 1.5 mg conjugate/ml

Equipment and materials:

Microplate washer
Microplate reader
Microplates: 96 wells plates Greiner cat. Number 655001
Reagent reservoirs
Multi-channel pipet (8 or 12 canals)

Procedure:

- Dilute the coating antibody 1000 times in coating buffer.
- Bring 200 µl of the antibody solution in the wells of the ELISA plate.
- Cover the plate with a lid and place the plate in a humid box (wet tissue on the bottom of the box). Close the box and incubate the box over-night in the refrigerator[1] or 3 hours at 37°C.

- Wash the plate in the plate washer with the washing buffer: 3 washings per plate[2].
- Prepare 2 dilutions of plant extract samples in SEB; undiluted sample and 1/10.
- Dilute the positive control 10 times in SEB.
- Bring 200 µl of the solutions in the wells of the ELISA plate. Fill 3 wells with the positive control and 3 wells with the negative control.
- Cover the plate with a lid and place the plate in a humid box. Close the box and incubate the box over-night in the refrigerator[1].

- Wash the plate in the plate washer with the washing buffer: 4 soakings and washings per plate.
- Dilute the AP conjugate 1000 times in SEB.
- Bring 200 µl of the conjugate solution in the wells of the ELISA plate .
- Cover the plate with a lid and place the plate in a humid box. Close the box and incubate the box over-night in the refrigerator[1] or 3 hours at 37°C.

- Wash the plate in the plate washer with the washing buffer: 4 soakings and washings per plate.
- Prepare the substrate.
- Bring 200 µl of the substrate in each well of the ELISA plate.
- Cover the plate with a lid and incubate the plate at room temperature until the positive control and positive samples are coloring yellow. This may take 15 minutes to several hours depending on the concentration of the virus in the samples and the reactivity of the antibodies.
- Read the plate in the plate reader after 60 minutes of incubation.

Foot-notes:

1. The incubation time is not critical. It is also possible to incubate during 4 hours at 27°C. It is recommended not to use high temperatures when incubating plant extracts.
2. The washing may also be performed by hand. Empty the plates with force and fill them several times with tap water of good quality or PBST. Soak the plates during 5 minutes with PBST and empty them again with force.