DAS-ELISA Method

Prepare coating antibody (MAb) in coating buffer at recommended dilution and dispense (200 µL) into wells of an ELISA plate.

Incubate 4hrs at 37^{o}C.

Wash plate x4 with wash buffer and blot dry.

Prepare sample by homogenising plant material in extraction buffer 1/10 (w/v) and dispense (200 µL) into test wells. Incubate overnight at 4^{o}C.

Wash plate x4 with wash buffer and blot dry.

Dilute antibody-AP conjugate at recommended dilution in extraction buffer and dispense (200 µL) into test wells. Incubate 2hrs 37^{o}C.

Wash plate x4 with wash buffer and blot dry.

Prepare substrate 1mg/mL in substrate buffer and dispense (200 µL) into test wells. Incubate at ambient for 1-2hrs.

Read OD of test wells at 405n.

Buffer Recipes

Coating buffer Substance	Amount
Na_2CO_3	0.8g
$NaHCO_3$	1.4g
1% NaN_3	10mL
H_2O	500mL
pH 9.6	

Wash buffer

Phosphate Buffered Saline + 0.05% Tween 20

Extraction buffer Substance	Amount
Polyvinyl pyrrolidone	10g
Bovine serum albumin	1g
1% NaN_3	10mL
Wash buffer	500mL
pH 7.4	

Substrate buffer Substance	Amount
Diethanolamine	48.5mL
1% Sodium azide	10mL
H_2O	500mL
pH 9.8	

1mg p-nitrophenyl phosphate per mL substrate buffer