LOEWE®

Instructions for ELISA: Standard Double Antibody Sandwich Assay (DAS-ELISA)

Assay Principle

During the first step of the assay the surface of a microtiter plate is coated with the antigen-specific coating-antibody (IgG). When an antigen-containing sample is added during the second step, the antigen binds to the immobilized IgG, forming an antibody-antigen complex. This complex reacts with the enzyme-labelled antibody-AP-conjugate during the third step by forming a double-antibody sandwich. During the fourth step the alkaline phosphatase (AP) reacts with the substrate 4-nitrophenylphosphate in an enzymatic reaction, resulting in yellow coloured 4-nitrophenol as product. This colour development can be evaluated visually or measured in a spectrophotometer at 405 nm after 1 and 2 hours.

Handling and Storage of the Reagents

Our DAS-ELISA reagents are standardized for use at a dilution of 1:200 and a test volume of 200 µl/well. The products must be kept refrigerated (ca. 4°C) upon receipt. Once opened, we recommend using the reagents within 5 months.

Assay procedure

	Steps	Dilution of Reagents	Add (per well)	Incubate	at	Wash*
1.	Application of coating-antibody (IgG)	Dilute IgG **1:200** from original vial in Coating Buffer	0.2 ml	4 h	37°C	4 x
2.	Sample application:	Prepare samples at a **1:20** dilution in Sample Buffer, if not stated otherwise in the product certificate. (Dilute LOEWE® **positive or negative controls in 2.1 ml** Sample Buffer, if not specified otherwise.)	0.2 ml	over night	4°C	4 x
3.	Application of antibody-AP-conjugate	Dilute AP-conjugate **1:200** from original vial in Conjugate Buffer	0.2 ml	4 h	37°C	4 x
4.	Enzymatic assay	Substrate Solution	0.2 ml	1 - 2 h	room temp.	-

***Washing:** After each incubation step, the reagents are removed with Wash Buffer by four washing cycles using an automated washer.

Buffer Formulations

Coating Buffer	1.59 g Na_2CO_3 2.93 g $NaHCO_3$	Dissolve in distilled water and fill to 1 l. Adjust pH 9.6. (Store refrigerated)
Wash Buffer	8.0 g NaCl 2.9 g Na_2HPO_4 x 12 H_2O 0.2 g KH_2PO_4 0.2 g KCl 0.5 ml Tween 20	Dissolve in distilled water and fill to 1 l. Adjust pH 7.2 - 7.4. (Store refrigerated)
Conjugate/Sample Buffer for sample preparation and conjugate dilution	Ingredients for Wash Buffer formulation (see above) *and add*: 20 g polyvinyl pyrrolidone (K10-K40) 2 g bovine serum albumin 0.1 g NaN_3 (only if desired)	Dissolve in distilled water and fill to 1 l. Adjust pH 7.4. (Store refrigerated for no longer than 1 week. We recommend freezing aliquots and using the buffer solution as fresh as possible.)
Substrate Buffer	97 ml diethanolamine 0.2 g $MgCl_2$ x 6 H_2O	Dissolve in distilled water and fill to 1 l. Adjust pH to 9.8 with 1 N HCl. (Store refrigerated)
Substrate Solution	1 mg/ml 4-nitrophenylphosphate-di-Na-salt in substrate buffer	Prepare this solution immediately prior to use!

Evaluation

We strongly advise to add positive and negative controls to the plate. To determine potential background of healthy plants, fresh non-infected extracts of the tested species, should be added to the plate. The positive/negative threshold needs to be determined by the user, as it depends on many factors, such as plant species and its physiological conditions (e.g. tissue type, age).