primediagnostics

Protocol Double antibody sandwich (DAS) ELISA

Our reagents are optimized for use in DAS-ELISA using certified NUNC-Immuno Plates Maxisorp F96 and operating with a working volume of 200 µl per well. The incubations are performed in a tightly closed humid box. During incubation the plates are covered with a lid or tape.
For washing in case you do not have an automatic washer: empty the wells in the sink, add washing buffer and soak the wells 15 seconds. Remove any liquid by blotting the plates on paper towels. Repeat this 3 – 5 times (see page 2).

Buffers and Chemicals:

- Coating buffer: 1.59 gr Na_2CO_3, 2.94 gr $NaHCO_3$, pH 9.6
  Add demineralized water to 1000 ml total volume.
- PBS 0.01 M: 8.18 gr NaCl, 0.15 gr KCl, 0.27 gr KH_2PO_4,
  1.42 gr Na_2HPO_4 x 2 H_2O, pH 7.4.
  Add demineralized water to 1000 ml total volume.
- Washing buffer (PBST): 0.05 % Tween-20 in PBS 0.01 M
- Extraction buffer (SEB): 0.2% egg ovalbumine (grade II), 2% PVP40,
  0.05% Tween-20 and 0.1 % Proclin 300 in PBS 0.01 M
- Substrate buffer: 97 ml diethanolamine, pH 9.8
  Add demineralized water to 1000 ml total volume.
- Substrate: 15 mg paranitrophenylphosphate (pNPP) in 20 ml substrate buffer

Procedure:

		Incubation buffer	Incubation time and temperature	Concentration of reagent
1	Coating	Coating buffer	Overnight at 4ºC or 3h at 37ºC	1000x diluted coating antibody
2	Controls and samples	Extraction buffer	Overnight at 4ºC	10x diluted positive control
3	Conjugate	Extraction buffer	Overnight at 4ºC or 3h at 37ºC	1000x diluted AP-conjugate
4	Substrate	Substrate buffer	30 min at room temperature	0.75 mg/ml pNPP

Sample Preparation:

Prepare a sample extract by crushing the plant material in extraction buffer; 0.1 gr plant material in 1 ml buffer. The resulting extract can be tested without a without further dilution, but it is recommended to test a 10 times dilution of the extract as well.
We recommend using negative controls existing of healthy plant extracts originating from the appropriate host of the pathogen as well as an internal control existing of SEB.
The positive control produced by Prime Diagnostics is a qualitative control and can be used as an internal control for the assay only. We recommend using an in-house positive control as well.

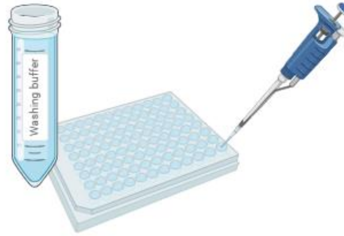
Remarks:

- A sample is positive if the ratio (OD_{405} sample/OD_{405} healthy plant extract) is at least 2.
- The use of other dilutions for the reagents will cause differences in reactivity, specificity, selectivity and detection limits.
- Lower reaction volumes will cause higher detection levels and lower ratio's.
- Different incubation times and temperatures will cause differences in sensitivity and background reactions.
- If the buffers are to be stored for more than 1 week it is recommendable to add Proclin 300 in a final concentration of 0.1%

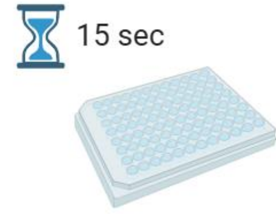
DAS-ELISA wash step

1. Empty the wells by holding the plate in your hand, and slamming it on a paper towel. Use force.

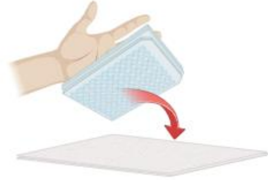
2. Add washing buffer to wells



3. Wait 15 seconds



4. Empty the wells by holding the plate in your hand, and slamming it on a paper towel. Use force.



5. Repeat step 2 to 4 three to five times

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