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: empty the wells and soak the wells 15 seconds with washing buffer while shaking. Repeat this 3 – 5 times. Remove any liquid by blotting the plates on paper towels.

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 0.05% NaN_3 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 minutes at room temperature	0.75 mg/ml pNPP

Sample preparation:
Prepare a sample extract in an appropriate buffer and test the extract without further dilution. 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 Sodium Azide in a final concentration of 0.05%.

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