Leibniz-Institut
DSMZ-Deutsche Sammlung von
Mikroorganismen und Zellkulturen GmbH

Triple Antibody Sandwich ELISA (TAS-ELISA)

Our ELISA reagents are optimized using greiner bio-one microplates, medium binding.
Before opening the tubes containing coating antibody (IgG), MAb and RAM-AP- Conjugate please spin down all the liquid by a short centrifugation (approx. 3000rpm for a few seconds)

1. Dilute specific antibody in coating buffer (recommended dilution see delivery note and tube); i.e. 20µl in 20 ml buffer at a recommended dilution of 1:1000 or 40µl in 20 ml buffer at a recommended dilution of 1:500. Add 200µl to each well of the microtiter plate.
2. Cover the plate and incubate at 37 °C for 2- 4 h.
3. Wash plate with PBS-Tween using wash bottle, soak for a few minutes and repeat washing two times. Blot plates by tapping upside down on tissue paper.
4. Add 200 µl of 2% skim milk in PBS-Tween to each well **(blocking)**.
   Cover the plate and incubate for 30 min at 37°C

5. Remove blocking solution and tap dry.
6. Extract samples 1:20 (w/v) in extraction buffer. Add 200 µl aliquots of the test sample to duplicate wells.
7. Cover the plate and incubate overnight at 4 °C.
8. Wash three times as in step 3.

9. Add 200 µl of the MAb in appropriate (recommended dilution see delivery note and tube label) conjugate buffer to each well.
10. Cover the plate and incubate at 37 °C for 2- 4 hours.
11. Wash three times as in step 3.

12. Dilute RAM-AP 1:1000 in conjugate buffer, i.e., 20µl in 20 ml buffer. Add 200 µl to each well.
13. Cover the plate and incubate at 37 °C for 1 hour.
14. Wash three times as in step 3.

Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
Plant Virus Department, Inhoffenstraße 7 B, 38124 Braunschweig, GERMANY
Tel.: +49(0)531 26 16-401/-402 Fax: +49(0)531 26 16-455 E-Mai: plantvirus@dsmz.de Internet: www.dsmz.de

15. Add 200 µl aliquots of freshly prepared substrate (1 mg/ ml para-nitrophenyl- phosphate in substrate buffer) to each well.

16. Cover the plate and incubate at 37°C for 30-60 min, or as long as necessary to obtain clear reactions

17. Assess results by:
    a) Visual observation
    b) Spectrophotometric measurement of absorbance at 405 nm

Reference

Clark, M. F. and Adams. A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475-483.

Buffers used in ELISA

1. ***Coating buffer (pH 9.6)***

   1.59 g sodium carbonate (Na_2CO_3)
   2.93 g sodium bicarbonate ($NaHCO_3$)
   0.20 g sodium azide (NaN_3)
   Dissolve in 900 ml H_2O, adjust pH to 9.6 with HCl and make up to 1 l.

2. ***PBS (pH 7.4) phosphate buffered saline***

   8.0 g sodium chloride (NaCl)
   0.2 g monobasic potassium phosphate (KH_2PO_4)
   1.15 g dibasic sodium phosphate (Na_2HPO_4)
   0.2 g potassium chloride (KCl)
   0.2 g sodium azide (NaN_3)
   Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 l.

3. ***PBS-Tween (PBST)***

   PBS + 0.5 ml Tween 20 per liter

4. ***Sample extraction buffer (pH 7.4)***

   PBST + 2% PVP (e.g. Serva PVP-15 polyvinyl pyrrolidone)

5. ***Sample extraction buffer (pH 8.5)for Begomoviruses***

   0.05 M Tris containing 0.06 M sodium sulfite, pH 8.5

6. ***Conjugate buffer***

   PBST + 2% PVP + 0.2% egg albumin (e.g. Sigma A-5253)

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7. ***Substrate buffer***

97 ml diethanolamine
600 ml H_2O
0.2 g sodium azide (NaN_3)
Adjust to pH 9.8 with HCl and make up to 1 liter with H_2O

Buffers can be stored at 4 ° C for at least 2 months. Warm to room temperature before use.

ELISA Troubleshooting

1. *No color development*

a) Did you omit any steps?
b) Did you use the correct buffer for each step?
c) Is your enzyme OK? Serum OK?
d) Is your positive control homologous to antiserum (IgG)?

Recommendations - Do a titration plate. Use a reliable positive control in each plate.

2. *Nonspecific color development*

a) If in edge wells only:
- Make sure the humidity in the incubator is sufficiently high.
- If this does not help, don't use edge or border wells, fill with buffer only.

b) If in whole plate:
- incomplete washing
- old substrate
- use recommended ELISA plate (greiner medium binding)
- error in loading sequence

Recommendations - Use reliable negative control in each plate.
Use fresh substrate and check for spontaneous color change. Cover plates while incubating. Check pH of the buffers used.

c) Some wells with inconsistent or unexpected reactions
- incomplete washing
- error in loading test antigens
- spillage between wells

Recommendations - Use extra wash step, handle plates carefully with lids on, use predetermined loading pattern before loading. Blot top of plate after rinsing.

3. *Color development very rapid; some color in healthy samples*

a) Enzyme conjugate concentration too high
b) Substrate concentration too high

Recommendations - Use enzyme conjugate and substrate concentrations that will give $OD_{405\ nm}$ of about 1.0 in 30 to 60 min with good antigen source.

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