DSMZ AS-1022 PepMV, Pepino Mosaic Virus
Double Antibody Sandwich ELISA (DAS-ELISA)

Our ELISA reagents are optimized using greiner bio-one microplates, medium binding. 
before opening the tubes containing coating antibody (IgG) and IgG-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 plates 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. Extract samples 1:20 (w/v) in extraction buffer. Add 200 µl aliquots of the test sample to 
duplicate wells.

5. Cover the plates and incubate overnight at 4 °C.

6. Wash three times as in step 3.

7. Add 200 µl enzyme conjugate, recommended dilution is given in the delivery note, in 
conjugate buffer.

8. Cover the plates and incubate at 37 °C for 2- 4 hours.

9. Wash three times as in step 3.

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

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

12. Assess results by: 
a) Visual observation  
b) Spectrophotometric measurement of absorbance at 405 nm
Buffers used in ELISA

1. Coating buffer (pH 9.6)

   1.59 g sodium carbonate (Na₂CO₃)
   2.93 g sodium bicarbonate (NaHCO₃)
   0.20 g sodium azide (NaN₃)

   Dissolve in 900 ml H₂O, 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 (KH₂PO₄)
   1.15 g dibasic sodium phosphate (Na₂HPO₄)
   0.2 g potassium chloride (KCl)
   0.2 g sodium azide (NaN₃)

   Dissolve in 900 ml H₂O, 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) for Pepino mosaic virus

   Sample extraction buffer (pH 7.4)
   PBST + 2% PVP (e.g. Serva PVP-15 polyvinyl pyrrolidone) +
   0.02 M Sodium sulfite (Na₂SO₃)

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)

7. Substrate buffer

   97 ml diethanolamine
   600 ml H₂O
   0.2 g sodium azide (NaN₃)

   Adjust to pH 9.8 with HCl and make up to 1 liter with H₂O

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 reliable positive control in each plate. Pretest enzyme conjugate on substrate.

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

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 OD405 nm of about 1.0 in 30 to 60 min with good antigen source.