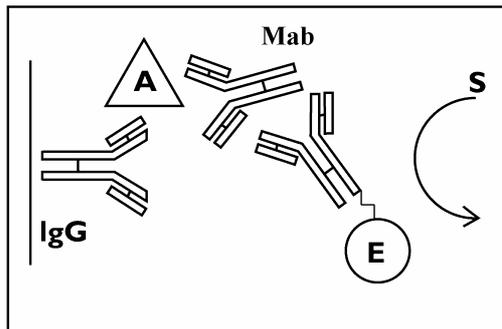


IDENTIKIT: Tas – general B

Assay Principle



The triple antibody sandwich ELISA (TAS) uses polyclonal antibodies (IgG) which are bound to the surface of the microtitre plate to capture the antigen (A) of interest and a monoclonal antibody (Mab) raised in rats to detect the antigen. An anti-species antibody-enzyme conjugate (E) is used to detect the monoclonal antibody. Alternatively, a monoclonal may be used to capture the antigen if both monoclonals are raised in different species.

Reagents supplied with ADGEN IDENTIKIT

12 x 8 well microtitre strips
One microtitre strip holder.
Extraction buffer (ten times concentrate)
Mab/Conjugate buffer (five times concentrate)
Probe Antibody
Anti-Rat IgG-AP conjugate
ADGEN Blue liquid substrate for the detection of AP
One vial of positive control
One vial of negative control
Three foil packs of phosphate buffered saline salts to make three litres of phosphate buffered saline (PBS)
One bottle of Tween 20

Allow all reagents to come to room temperature before commencing the test.

Other Items Required

Pipette for 1 - 100 μ l volumes
Pipette for 1000 μ l volumes
Pipette tips suitable for the above pipettes
37 $^{\circ}$ C incubator
Wash bottle/plate washer
Plate reader with a 405nm filter (for ADGEN Yellow) or 595 - 650nm (for ADGEN Blue)
Paper towelling
Sandwich box
Mortar and pestle (or alternative)

Preparing for the ADGEN IDENTIKIT assay

1. Remove the required number of strips from the bag.

ADGEN recommend testing all samples and controls in duplicate. Remove the number of strips that are required for the assay from the foil bag and place in the strip holder. The strips will only fit into the holder one way. If more than one strip is being used the strips should be labelled at one end with a number corresponding to the strip. Return the unused strips to the foil bag with the desiccant and seal the bag with tape.

2. Dilution of Extraction buffer

For 10 samples dilute 10ml of **Extraction buffer concentrate** with 90ml of deionised/distilled water.

3. Preparation of Samples

Grind 1g of plant material with 10ml of **working strength extraction buffer** in a mortar and pestle (or alternative method of grinding). In some cases the ratio of sample to buffer may be reduced to obtain a cleared signal if the plant material is not highly infected. However, in most cases 1g/10ml should be sufficient. Filter the samples through a layer of muslin (or similar fine cotton gauze), if this is not available then allow the plant material to settle and use the supernatant in the test. There is sufficient **extraction buffer** to allow extraction of 50 samples at this ratio. The samples should then be tested in duplicate wells.

4. Preparation of Controls

Add 2ml of deionised/distilled water to the positive and negative control and shake gently to dissolve the contents. Any unused reconstituted control may be stored at -20 $^{\circ}$ C however, the performance of the positive controls may decrease when stored in this manner.

5. Addition of samples/controls to the wells.

Carefully pipette 100 μ l of each sample, positive and negative control in to duplicate wells in the well holder. Wrap the plate tightly in cling film or place in a plastic box with some damp paper towels and close the box. Incubate overnight at 4 $^{\circ}$ C or for 3-4 hours at 37 $^{\circ}$ C.

6. Preparing the wash buffer.

Dissolve the contents of one foil pack of PBS with deionised/distilled water and make up to 1 litre. Add 0.5ml of Tween 20 to the prepared PBS. Mix well to ensure that the Tween 20 is in solution. This is the **wash buffer**.

7. Washing the microtitre strips.

Remove the plate from the incubator and wash with **wash buffer**. To do this fill the wells of the plate with **wash buffer** and invert to remove the buffer. **Repeat this twice**, then pat the plate dry on paper towels.

8. Preparing the conjugate buffer.

The conjugate buffer is used to dilute both the probe antibody and the conjugate. Dilute the concentrated **conjugate buffer** with deionised/distilled water. That is, for every 1ml of conjugate required for the test dilute 200 μ l of **conjugate buffer concentrate** with 800 μ l of water.

9. Preparing the probe antibody.

Add 550 μ l of **working strength conjugate buffer** to the tube containing the **probe antibody**. Ensure that the contents of the vial are well mixed. This stock is stable for 2-4 weeks if stored at 4 $^{\circ}$ C. The probe antibody stock should be further diluted 1/20 with **conjugate buffer** before use. That is, for every 1ml of **probe** required in the assay add 50 μ l of the stock to 950 μ l of **conjugate buffer**.

10. Washing the microtitre strips.

Wash as in (7) above.

11. Addition of probe antibody to the microtitre wells.

Carefully pipette 100µl of the **probe** into each of the wells in the well holder. Wrap the plate tightly in cling film or place in a plastic box with some damp paper towels and close the box. Place the plate/box in a 37°C incubator and leave for 2 hours.

12. Preparing the conjugate.

Add 550µl of **working strength conjugate buffer** to the tube containing the conjugate. Ensure that the contents of the vial are well mixed. This stock is stable for 2 - 4 weeks if stored at 4°C. The conjugate stock should be further diluted 1/20 with **conjugate buffer** before use.

13. Washing the microtitre strips.

Wash as in (7) above.

14. Addition of the conjugate to the microtitre wells.

Carefully pipette 100µl of the **conjugate** into each of the wells in the well holder. Wrap the plate tightly in cling film or place in a plastic box with some damp paper towels and close the box. Place the plate/box in a 37°C incubator and leave for 1 hour.

15. Washing the microtitre strips.

Remove the plate from the incubator and wash with **wash buffer**. To do this fill the wells of the plate with **wash buffer** and invert to remove the buffer repeat this **three times**, then pat the plate dry on paper towels. An extra washing step is included at this stage to ensure that all unbound conjugate is removed from the wells, this is important to prevent the occurrence of false positive results (see **Trouble Shooting Guide**).

16. Preparing ADGEN Blue liquid Substrate.

Using a clean pipette tip carefully pipette the same amount of substrate from each bottle into a clean tube and gently mix. The substrate should be protected from light. The substrate should be prepared no more than 30 minutes before use.

17. Addition of ADGEN Blue liquid Substrate to the microtitre wells.

Carefully pipette 100µl of the **substrate** into each of the wells in the well holder. Wrap the plate tightly in cling film. Incubate in the dark at room temperature for up to one hour. The sensitivity of this substrate allows this stage to be reduced for some applications.

18. Interpretation of Results.

Read the absorbance using a spectrophotometer at 620nm. Alternatively, positive and negative values may be scored visually although this may not be as accurate as using a spectrophotometer. A positive sample may be determined as one which gives an absorbance value which is greater than the absorbance value of the negative control. A negative sample is one which gives an absorbance value which is the same as, or less than, the negative control. Visually, a positive sample will give a darker colour than the negative control and a negative sample will give a similar, or lighter, colour to the negative control.

Stability of Buffers and Solutions

Stock solutions of buffers are stable for at least 1 year. Diluted buffers should be stored at 4°C and will be stable for 3 - 4 weeks. The addition of sodium azide (0.01%) to diluted buffers will increase their stability and shelf life.

Stock solutions of antibodies/conjugates are stable for between 6 - 12 months. Diluted antibodies/conjugates are stable for a few days if stored at 4°C. However, we recommend that only sufficient antibody/conjugate is prepared to meet your immediate requirements.

Trouble Shooting Guide

A uniform high level of colour appears in all wells.

- The plate may not have been washed properly.
- The substrate solution may have been contaminated. pNPP tablets can be contaminated by touching them, using ADGEN Blue removes this potential source of contamination.
- The substrate solution may have been exposed to bright light.
- One of the buffers may have been contaminated by conjugate.

All wells are coloured but to different degrees

- The plate may not have been properly washed.
- The plate may have been exposed to bright light.

Colour appears in all of the outer wells of the plate

- The outer wells may not have been properly washed.
- You may be experiencing 'edge effects'. This is a problem associated with the cooling procedure employed when the plates are being moulded. Newer, high quality plates such as those used at, and supplied by, ADGEN do not cause this problem.

Some colour appears in a negative control well, while some of the other wells are clear.

- A positive sample may have been added to the negative control well.
- During washing some positive control sample may have been washed into the negative control well.
- Contamination of the negative control well may have occurred by carry over of a positive sample if the same pipette tip was used. New tips must be used for every sample.

All of the wells, including the positive control, are clear.

- The antibody-enzyme conjugate may not have been added to the conjugate buffer.
- The positive control may have gone off.
- The pNPP substrate tablet may not have been added to the substrate buffer.

The colour in the positive control wells is very low

- One of the buffers that have been used may be too old.
- The positive control may be starting to go 'off'.
- The substrate buffer may have been diluted with PBST and not water as recommended. Phosphate in PBST reduces the amount of colour produced.

If you still have a problem then prompt and comprehensive technical advice is always available from ADGEN.

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