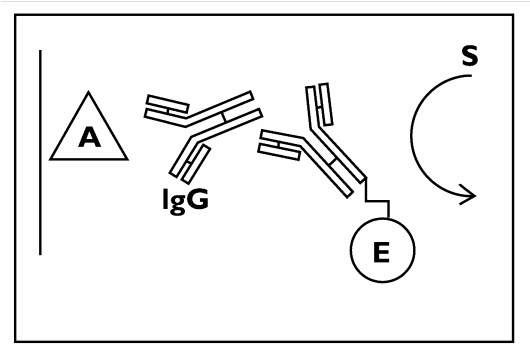
IDENTIKIT: pta general Y

Assay Principle



In a plate trapped antigen ELISA the sample of interest (A) is coated on to the microtitre plate. After a period of incubation the unbound sample is removed by washing, any free sites on the plate are blocked by the addition of a blocking buffer containing a high proportion of protein. The blocking buffer is removed by washing and an antibody (IgG) which has been raised against the antigen is added to the plate. After washing, a second antibody which is conjugated to enzyme (E) - in our case alkaline phosphatase - is added to the plate and incubated. The secondary antibody is specific for the species that the primary antibody is raised in. The unbound antibody-enzyme conjugate is washed from the plate and the substrate (S) for the enzyme is added. If there is any bound antibody-enzyme conjugate the substrate will change colour.

Reagents supplied with ADGEN IDENTIKIT

12 x 8 well microtitre strips
One microtitre strip holder.
Coating buffer (ten times concentrate)
Conjugate buffer (five times concentrate)
Probe Antibody
Anti-species IgG-AP conjugate
ADGEN Yellow liquid pNPP substrate
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 - 100µl volumes
Pipette for 1000µl volumes
Pipette tips suitable for the above pipettes
37^0C 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 bag and place in the strip holder. 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 bag and seal the bag with tape.

2. **Dilution of Coating Buffer**

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

3. **Preparation of Samples**

   Grind 1g of plant material with 10ml of **working strength coating 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 **coating buffer** to allow extraction of 50 samples at this ratio. The samples should then be tested in duplicate wells.

4. **Preparation of Controls**

   Add 1ml of coating buffer to the positive control and shake gently. Add 2ml dH_2O to the negative control and shake to resuspend.

5. **Addition of samples/controls to the wells.**

   Carefully pipette 100µl 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 4^0C.

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. **Addition of Blocking Buffer**

   The blocking buffer should be diluted in the prepared wash buffer by adding 21ml to the contents of the blocking buffer vial. 200µl of blocking buffer is added to the wells after washing. The plate should be incubated for at least 1 hour at 37^0C (although longer incubation times at this stage will not interfere with the assay).

9. **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 200µl of **conjugate buffer concentrate** with 800µl of water.

10. **Preparing the probe antibody.**

Add 550µl 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 4^0C. 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 50µl of the stock to 950µl of **conjugate buffer.**

11. Washing the microtitre strips.

Wash as in **(7)** above.

12. 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 37^0C incubator and leave for 1 hour.

13. 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 4^0C. The conjugate stock should be further diluted 1/20 with **conjugate buffer** before use.

14. Washing the microtitre strips.

Wash as in **(7)** above.

15. 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 37^0C incubator and leave for 1 hour.

16. Washing the microtitre strips.

Wash as in **(7)** above. Include one extra wash step to ensure that the unbound conjugate is completely removed.

17. Preparing ADGEN Yellow liquid Substrate.

Using a clean pipette tip carefully pipette the required amount of substrate from the bottle into a clean tube. The substrate should be protected from light.

18. Addition of ADGEN Yellow 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 1 hour.

19. Interpretation of Results.

Read the absorbance using a spectrophotometer at 405nm. 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 4^0C 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 4^0C. 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 Yellow 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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