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A Geno Technology, Inc. (USA) brand name

Quantitative Precipitin Assay (QPA)

Teacher's Guidebook

(Cat. # BE-505)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 vial Fluorescent Antigen
- 1 vial Antibody: BE Antibody 1
- 1 bottle PBS
- 1 bottle Solubilization Buffer
- 50 Centrifuge Tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Store Fluorescent Antigen and BE Antibody 1 at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

ADDITIONAL EQUIPMENT REQUIRED

- Low Speed Centrifuge for 1.5-2ml tubes
- UV light box
- Fluorescence Reader (optional)

TIME REQUIRED

- Day 1: 60 minutes
- Day 2: 20-30 minutes

OBJECTIVES

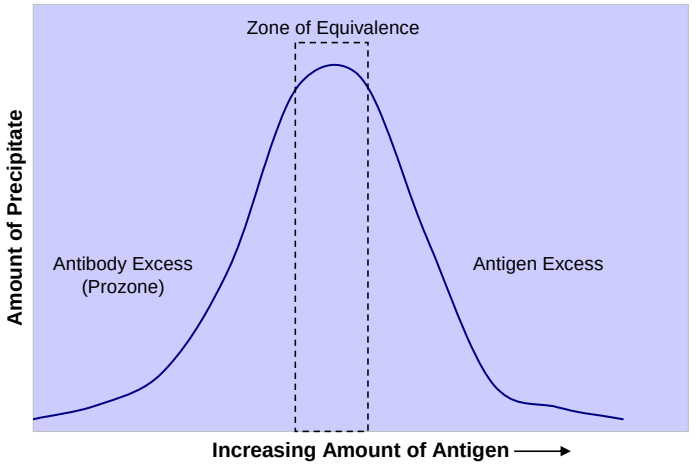
- Teaches principle of antibody and antigen interaction.
- Understand the principle of antibody:antigen precipitation.
- Quantitate ideal antibody:antigen concentrations.

BACKGROUND

The Quantitative Precipitin Technique is a simple technique that is routinely used in the analysis of antibody and antigen interactions and for the estimation of the antibody or antigen content in a sample. The technique is based on the interaction of antibody and antigen to form a large protein complex that in certain solutions (buffer) will result in precipitation.

One of the first observations of antigen-antibody interactions was their ability to precipitate when combined in certain proportions. The quantitative precipitin test relies on this fact. This test forms the basis of all quantitative studies of antigen-antibody interactions. Increasing amounts of antigen are added to a constant amount of antibody and the amount of precipitate formed in each tube is determined.

The precipitin test occurs only with multivalent antigens and is dependent on electrolyte concentration, pH, temperature, and the relative concentrations of antigen and antibody, the amount of precipitate formed increasing to a maximum and then decreasing as the relative antigen concentration is increased. By using increasing amounts of either antibody or antigen, scientists can get a good estimation of the ideal ratio of antibody and antigen, by plotting a graph of amount of precipitate versus antibody or antigen concentration. The ideal concentration is known as the zone of equivalence (see graph below).



Students undertake a simple experiment to determine the zone of equivalence of an antigen and antibody interaction. The kit is provided with a fluorescent-labeled protein to make visualization of the results clearer and easier to compare.

TEACHER'S PRE EXPERIMENT SET UP

Always wear gloves and protective clothing throughout the whole experiment.

1. Add 650µl PBS to the vial-containing Fluorescent Antigen. Soak the antigen for 5 minutes with periodically vortexing to dissolve the antigen completely.

2. Label six tubes with "Antigen" and aliquot 100µl antigen solution into each tube; supply each group with one tube of "Antigen".

3. Add 1.25ml PBS to the vial of BE Antibody 1. Soak the antibody for 5 minutes with periodically vortexing to dissolve the antibody completely.

4. Label six tubes with "Antibody" and aliquot 200µl antibody solution into each tube. In addition, add 200µl PBS to each tube. Briefly mix and supply each group with one tube of "Antibody".

5. Aliquot PBS and Solubilization buffer for each student group according to the next section.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components will be shared by the whole class and should be kept on a communal table.

- 100µl Antigen (0.25mg/ml)
- 400µl Antibody
- 1ml PBS
- 1ml Solubilization Buffer
- 8 Centrifuge Tubes (1.5ml)

PROCEDURE

Always wear gloves and protective clothing throughout the whole experiment.

1. Label seven tubes 2 to 8. Tube 1 is the original antigen tube.

2. Prepare serial dilutions of the antigen. Add 50µl PBS to tubes 2-8. Transfer 50µl Antigen solution from tube 1 to tube 2. Pipette up and down or vortex to mix.

3. Transfer 50µl from tube 2 to tube 3, mix as before. Continue transferring from tube 3 to 4, then 4-5 etc.

4. Once tube 8 is mixed, remove 50µl from tube 8 and discard.

5. Add 50µl Antibody solution to tubes 1-8 and mix as before.

6. Incubate all tubes at 37°C for an hour and then overnight at 4°C to allow for precipitation.
   If 37℃ incubator is not available then simply incubate at 4 ℃ overnight.

7. Next day, centrifuge all tubes at 5,000xg for 10 minutes. Carefully remove all supernatant without disturbing the pellets.
   The pellets are very small, white and translucent and are sometimes difficult to see. Note the orientation of the centrifuge tubes in the centrifuge to aid visualization of the pellets.

8. Wash pellet by adding 50µl PBS to each tube. Centrifuge all tubes at 5,000xg for 10 minutes. Carefully remove and discard all supernatant without disturbing the pellets.

9. Examine the pellets and record their size relative to each other. If a UV lamp or UV transilluminator is available, examine the pellets and record their sizes under UV light. (Note-best visibility is obtained with the use of a UV transilluminator).
   Visualizing the pellets with a UV lamp significantly improves their clarity and makes comparison easier.

10. Plot a graph of Relative size of pellet against tube number.

11. *OPTIONAL: If a fluorescence or UV reader is available, resuspend the pellet in 100µl Solubilization Buffer. Close the caps and vortex the tubes until the pellets are completely dissolved.*

12. *Observe the amount of precipitated fluorescent antigen under a UV light.*

13. *The fluorescence can be measured with a fluorescence reader (485/530nm) or the amount of protein can be measured with a UV spectrophotometer (280nm). Transfer the solution of each tube to a well of 96-well plate. Transfer 100µl PBS to a well as blank. Read fluorescence or UV absorbances*

14. *Draw a plot of fluorescence/UV absorbance values against antigen concentration.*

RESULTS, ANALYSIS & ASSESSMENT

1. Insert the antigen concentration of each tube and the relative pellet size in the table below:

Tube #	1	2	3	4	5	6	7	8
Antigen Conc. (mg/ml)								
Relative pellet size								

2. Plot a curve of relative pellet size (or fluorescence/ UV intensity) against tube # and antigen concentration.

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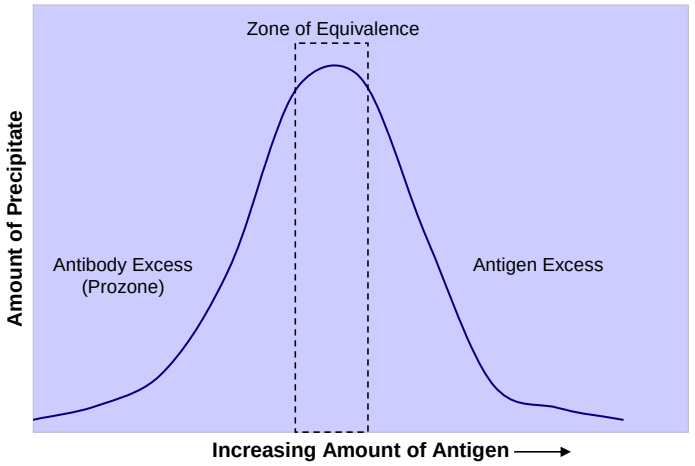

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