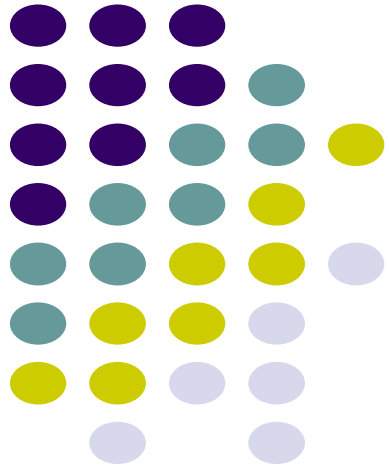
Immunology/antibody based techniques



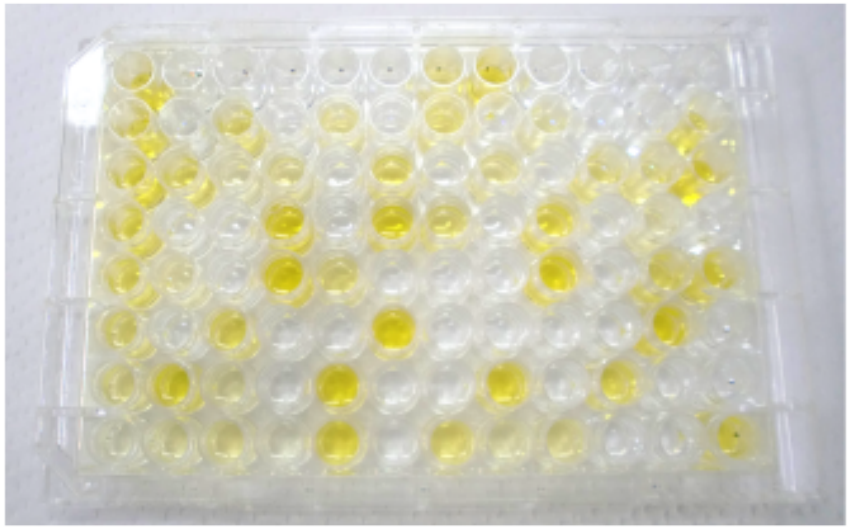
Serological assays

- Plant pathogens such as viruses cannot be cultivated ad hoc. Hence, serological assays were developed to detect them. More than a thousand other pathogens, bacteria, and fungi can now be detected using polyclonal and monoclonal antisera and techniques such as:
  - enzyme linked immunosorbent assay (ELISA),
  - western blots,
  - immunostrip assays,
  - dot-blot immune-binding assays, and
  - serologically specific electron microscopy (SSEM)
- Among them, ELISA, first employed in the 1970s, is by far the most widely used immunodiagnostic technique because of its high throughput potential. The sensitivity of ELISA varies depending on the organism, sample freshness, and titre;
- For instance, bacteria can be detected at 100 cfu mL^{-1}. Antibodies need to be stored at lower than −20 °C and should not be defrosted several times.

ELISA (Enzyme-linked immunosorbent assay)

- ELISA is a molecular method for identification of diseases based on antibodies and color change in the assay.
- In this method, the target epitopes (antigens) from the viruses, bacteria and fungi are made to specifically bind with antibodies conjugated to an enzyme.
- The detection can be visualized based on color changes resulting from the interaction between the substrate and the immobilized enzyme.

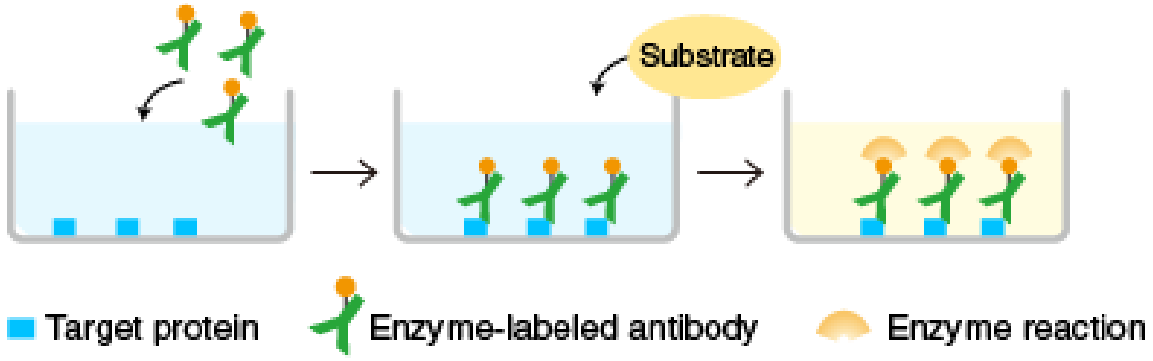
ELISA: An example of an assay using a 96-well plate.



The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.

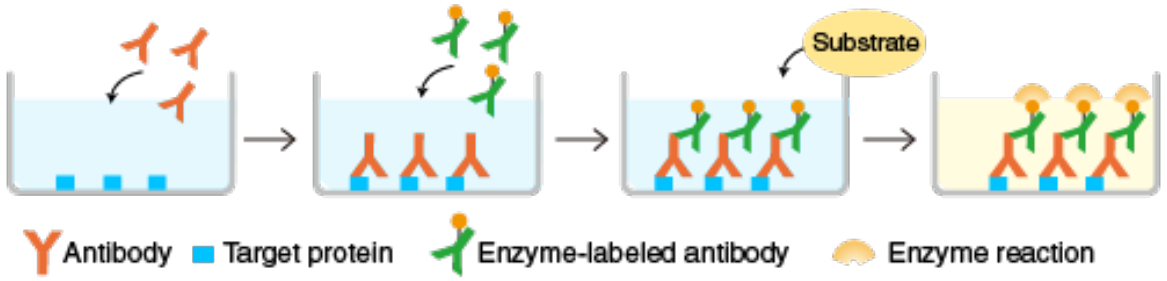
The principle

- Depending on the antigen-antibody combination, the ELISA assays can be classified as
  - direct ELISA,
  - indirect ELISA,
  - sandwich ELISA, and
  - competitive ELISA
- **Direct ELISA**
- A target protein (or a target antibody) is immobilized on the surface of microplate wells and incubated with an enzyme-labeled antibody to the target protein (or a specific antigen to the target antibody). After washing, the activity of the microplate well-bound enzyme is measured.



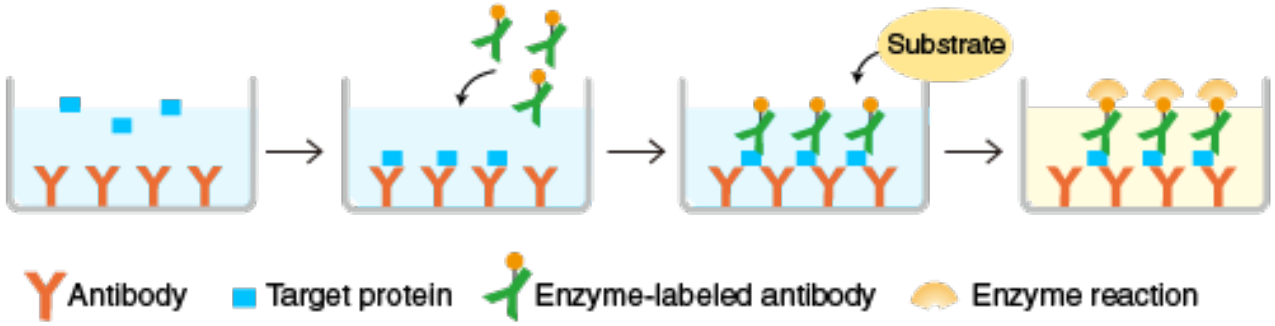
Indirect ELISA

- A target protein is immobilized on the surface of microplate wells and incubated with an antibody to the target protein (the primary antibody), followed by a secondary antibody against the primary antibody. After washing, the activity of the microplate well-bound enzyme is measured.
  Although indirect ELISA requires more steps than direct ELISA, labeled secondary antibodies are commercially available, eliminating the need to label the primary antibody.



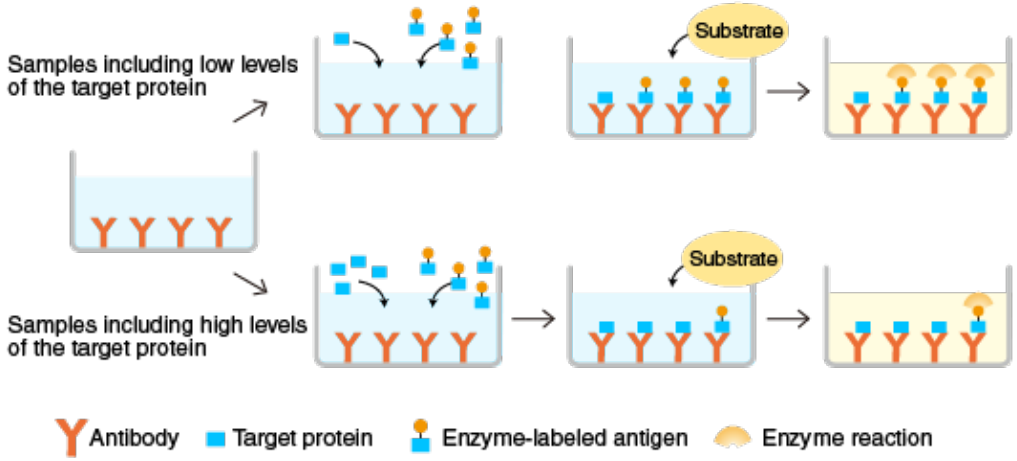
Sandwich ELISA

- An antibody to a target protein is immobilized on the surface of microplate wells and incubated first with the target protein and then with another target protein-specific antibody, which is labeled with an enzyme. After washing, the activity of the microplate well-bound enzyme is measured.
- The immobilized antibody (orange) and the enzyme-labeled antibody (green) must recognize different epitopes of the target protein. Compared to direct ELISA, the sandwich ELISA (combining antibodies to two different epitopes on the target protein) has a higher specificity. Sandwich ELISA is useful for applications that require a high accuracy.



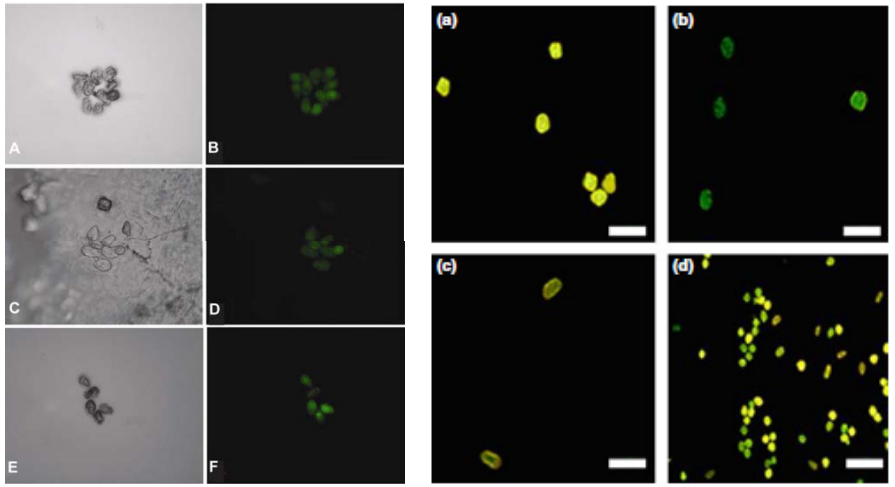
Competitive ELISA

- An antibody specific for a target protein is immobilized on the surface of microplate wells and incubated with samples containing the target protein and a known amount of enzyme-labeled target protein. After the reaction, the activity of the microplate well-bound enzyme is measured.
- When the antigen level in the sample is high, the level of antibody-bound enzyme-labeled antigen is lower and the color is lighter. Conversely, when it is low, the level of antibody-bound enzyme-labeled antigen is higher and the color, darker.
- When a target antigen is a small molecule, such as histamine, pesticide, and dioxin, two antibodies cannot simultaneously bind to the antigen in sandwich ELISA. Competitive ELISA is useful for the measurement of low molecular weight targets.



Immunofluorescence (IF)

- It is a fluorescence microscopy-based optical technique used for detection of pathogen infections in plant tissues. For this technique, plant samples are fixed to microscope slides in thin tissue sections. Detection is achieved by conjugating a fluorescent dye to the specific antibody to visualize the distribution of target molecule throughout the sample.
- IF has been used to detect onion crop infection by a fungus *Botrytis cinerea*. IF has also been combined with other techniques such as FISH for detection of crown rot pathogen *Dickeya* species in potatoes, an emerging disease in Europe.



Flow Cytometry (FCM)

- It is a laser-based optical technique widely used for cell counting and sorting, biomarker detection and protein engineering.
- Although FCM has been primarily applied to study cell cycle kinetics and antibiotic susceptibility, to enumerate bacteria, to differentiate viable from non-viable bacteria, and to characterize bacterial DNA and fungal spores, it is still a relatively new technique for plant disease detection application.