ELISA

- Enzyme-linked Immunosorbant Assay
- Detects the presence of minutes quantities of either an antibody or an antigen
- Important diagnostic tool in many areas of science and industry

Basics of ELISA

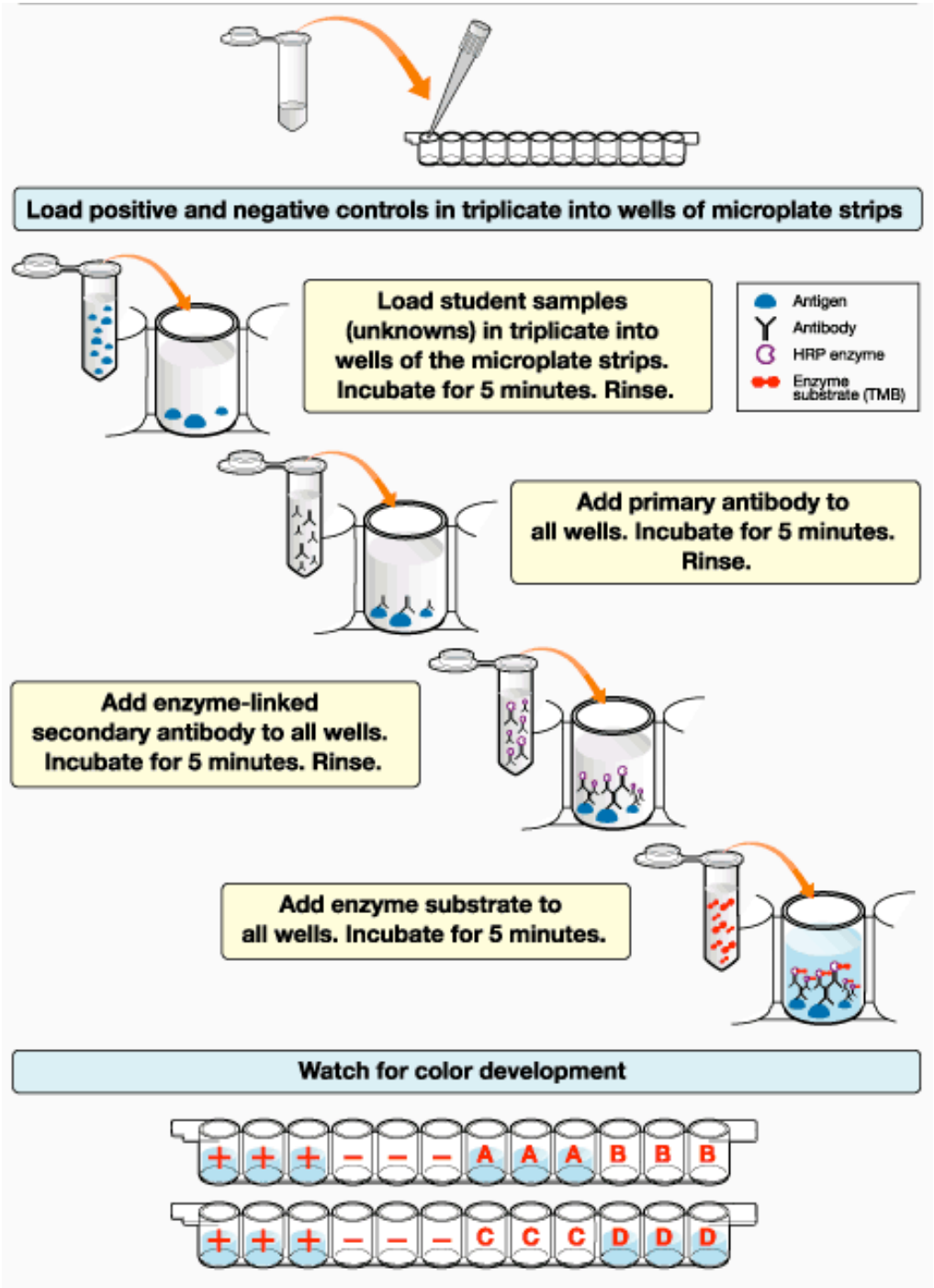
- The solution to be tested is “unknown” in that it might or might not contain the substance (antigen) of interest.
- Samples of the unknown solution are placed into small wells (usually polystyrene).
- Particles (including the antigen, if present) adsorb to the surfaces of the wells.

Basics of ELISA (cont.)

- Primary antibodies (specific for the antigen of interest) are added to the wells.
- Primary antibodies will attach only to the antigen; if no antigen is present, the primary antibodies will be washed away.
- Secondary antibodies (specific for the primary antibodies) are added to the wells.
- Secondary antibodies are linked to enzymes.

Basics of ELISA (cont.)

- Secondary antibodies will remain only if primary antibodies (and therefore antigens) are present.
- Substrate (specific for the linked enzyme) is added.
- If secondary antibodies are present, their enzymes will convert the substrate to some product.
- If product is detected, it indicates that antigen was present in the unknown solution.


Load positive and negative controls in triplicate into wells of microplate strips
Load student samples (unknowns) in triplicate into wells of the microplate strips. Incubate for 5 minutes. Rinse.
Antigen
Antibody
HRP enzyme
Enzyme substrate (TMB)
Add primary antibody to all wells. Incubate for 5 minutes. Rinse.
Add enzyme-linked secondary antibody to all wells. Incubate for 5 minutes. Rinse.
Add enzyme substrate to all wells. Incubate for 5 minutes.
Watch for color development
+ + + – – – A A A B B B
+ + + – – – C C C D D D