I. Protein Gel Electrophoresis

Gel electrophoresis = separation in an electric field
often SDS-PAGE = SDS polyacrylamide gel electrophoresis,

--- a negatively charged detergent (SDS) binds the protein,
# SDS molecules is roughly proportional to # of amino acids,
and the now uniformly negatively charged proteins (anions)
are separated by their molecular weights

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis is a technique that is used to separate proteins in a mixture for the purpose of identifying them individually. Proteins coated in SDS (which gives them a negative charge), are pulled through a thin gel by an electrical current. They separate on the gel based on size—the larger the protein, the slower it moves. Proteins can be stained with Coomassie Blue Dye for visualization or they can be detected by the Western Blotting technique.

II. Western Immunoblot Analysis

Western Immunoblot analysis allows researchers to determine the molecular weight of a protein and measure relative amounts of a protein present in different samples. The proteins are separated via SDS–PAGE, then transferred, or blotted, onto nitrocellulose paper or a membrane, retaining the same placement as on the gel. The blot is incubated with a generic protein to bind
the remainder of the paper. Then, an antibody that specifically binds to a particular protein is added to the solution. This antibody will have an enzyme attached to it that cannot be detected at this time (usually horseradish peroxidase). A colorless substrate will then be added that the enzyme attached to the antibody can convert to a colored product, thus revealing the location of the antibody–conjugated protein of interest.
III. Antibody Generation
Many of the antibodies used in immunochemical techniques are raised by repeated immunization of a suitable animal, e.g., rabbit, goat, donkey, or sheep, with a suspension of the appropriate antigen. One characteristic of large antigen molecules is that they induce the activation of many antibody-producing B cell clones in the immunized animal. This polyclonal mixture of resulting antibodies may then recognize a variety of epitopes on the antigen. A homogeneous population of antibodies (i.e. monoclonal antibodies) can be raised by fusion of B lymphocytes with immortal cell cultures to produce hybridomas. Hybridomas will produce many copies of the exact same antibody.

IV. Immunostaining
The antibodies used for specific detection can be polyclonal or monoclonal. Monoclonal antibodies are generally considered to exhibit greater specificity. Polyclonal antibodies are made by injecting animals with peptide antigens, and then after a secondary immune response is stimulated, isolating antibodies from whole serum. Thus, polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes.
Antibodies can also be classified as primary or secondary reagents. Primary antibodies are raised against an antigen of interest and are typically unconjugated (unlabelled), while secondary antibodies are raised against primary antibodies. Secondary antibodies recognize immunoglobulins of a particular species and are conjugated to either biotin or a reporter enzyme such as alkaline phosphatase or horseradish peroxidase. Some secondary antibodies are conjugated to fluorescent agents, such as the Alexa-Fluor family. The indirect method of detection involves an unlabeled primary antibody (first layer) that reacts with tissue antigen, and a labeled secondary antibody (second layer), which reacts with the primary antibody. (The secondary antibody must be against the IgG of the animal species in which the primary antibody has been raised.) This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody. The second layer antibody can be labeled with a fluorescent dye or an enzyme.