Western Blot

Proteins are heated in SDS that denatures the protein. SDS binds the length of the protein making it negatively charged. The protein is then run on a polyacrylamide gel towards the positive pole. After the gel runs it is electroblotted onto a membrane (often PVDF). The immobilized proteins are incubated with the primary antibody that recognizes the protein of interest. Then a secondary antibody (which is conjugated to an enzyme) is used to bind to the primary antibody. A substrate is added to the blot to allow the visualization of the antibody complex.