IEF separates proteins according to the pI (samples may be implemented under native or denaturing conditions). The method offers very high resolution and it is often used to provide information of presence of closely related derivatives (e.g., des-amido forms) or presence of glycosylated derivatives of the target protein. 2DE separates proteins according to the protein's pI (first dimension) and its molecular weight (second dimension). The method is a combination of IEF and SDS-PAGE. The resulting coordinate (pI, molecular weight) provides a unique identification of the protein. Differences in PTMs (e.g., phosphorylation) will often result in separate spots (slightly different pI and molecular weight). CE offers similar separation technologies. The CE methods can be used as a purity analysis. In native electrophoresis, proteins are separated according to charge, shape, and molecular weight in the absence of denaturants, ampholytes, or other reagents, which can influence the molecular properties or the electric field. The sample is typically transferred to or solubilized in 5% (w/v) sucrose or dilute gel buffer (1–5 mM). The gel pH and the pI of the protein(s) to be analyzed must match, as the net charge of the protein(s) may change from positive to negative or negative to positive affecting the protein's ability to enter the gel (i.e., if gel pH < protein pH, the protein will have a net positive charge; if gel pH > protein pH, the protein will have a net negative charge). Note that severe solubility problems can be experienced for certain proteins in the absence of denaturing and solubilizing agents such as urea or SDS. IEF standards can be used for native electrophoresis as well. Coomassie blue and silver staining are the two most common staining methods used for band detection on slab gels. Coomassie staining has a sensitivity of 0.05 to 0.5 pg protein per band. Silver staining is about 10–100 times more sensitive at enabling detection of 1–5 ng of protein per band. Native electrophoresis is used as a target protein identity method and to evaluate product-related impurities (e.g., des-amido forms, oxidized forms) during process development. One should, in general, be careful to use electrophoretic methods as purity analyses due to difficulties in quantifying the method.

*5.2.4.4 Western blot (WB)* WB (sometimes called protein immunoblot) is used to detect specific proteins; it uses gel electrophoresis to separate native proteins by 3D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane (typically nitrocellulose or polyvinylidene fluoride), where they are stained with antibodies specific for the target protein. The gel electrophoresis step is included in WB analysis to resolve the issue of the cross-reactivity of antibodies. There are now many reagent companies that specialize in providing antibodies (both monoclonal and polyclonal antibodies) against tens of thousands of different proteins. Commercial antibodies can be expensive, although the unbound antibody can be reused between experiments. This method is used in the fields of molecular biology, immunogenetics, and other molecular biology disciplines.