

Read this chapter to learn more about these topics:

Topic	See page
Analysis on a Western Blot	3.2
Analysis by Immunofluorescence Microscopy	3.13

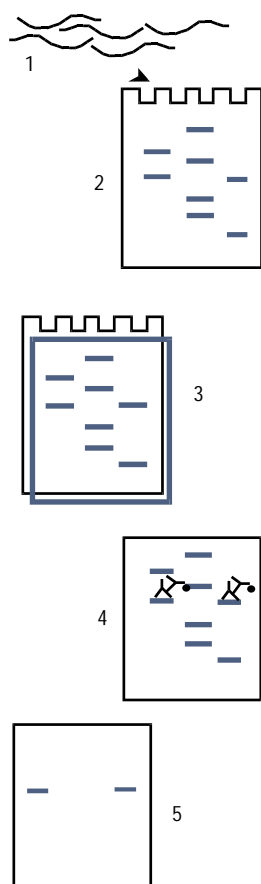
# Analysis of Tagged Proteins

3

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## Section 3A

### Analysis on a Western Blot



**Figure 3A.1: Steps in a Western blot.**  
The numbered steps are:

1. apply protein samples to gel;
2. separate the proteins electrophoretically;
3. blot the proteins from a gel onto a flexible membrane;
4. probe the membrane with a primary antibody that recognizes a specific (target) protein (followed in some cases by a secondary antibody that recognizes the primary antibody);
5. visualize the antibody-antigen complexes with an indicator molecule that produces a visible signal.

### Overview of technique

Western blot analysis is the most commonly used immunochemical technique for detection of epitope-tagged proteins. It allows the detection of epitope-tagged proteins in complex mixtures such as cell or membrane extracts (for instance, Canfield and Levenson, 1993; Duden *et al.*, 1991). When combined with immunoprecipitation (as described in Section 4.D of this manual), it can reveal information about the interaction of the tagged protein with other cell components (for instance, as in Dietzen, Hastings and Lublin, 1995).

In a Western blot, proteins are electrophoretically separated on an acrylamide gel, then transferred to a membrane detected with one or more antibodies (Figure 3A.1). The antibody detection technique may be:

- ▶ **Direct:** The membrane is incubated with an enzyme-conjugated tag-specific antibody. [Antibodies for direct detection are often conjugated with horseradish peroxidase (POD) or alkaline phosphatase (AP).]
- ▶ **Indirect:** The membrane is incubated first with an unconjugated tag-specific antibody (primary antibody), then with an enzyme-conjugated antibody (secondary antibody) that recognizes the tag-specific antibody. (Secondary antibodies for indirect detection of primary antibodies are usually conjugated with POD or AP.)

Suitable enzyme substrates for Western blotting must produce a signal on the membrane at the site of the enzyme-conjugated antibody (and thus, the tagged protein). Examples of Western blotting substrates include:

- ▶ **Chemiluminescent Western Blotting Substrate (POD)** for chemiluminescent visualization of peroxidase-conjugated antibodies (Signal recorded on X-ray film)
- ▶ **BM Teton** for chromogenic visualization of peroxidase-conjugated antibodies (Signal recorded on the membrane)

- ▶ **CDP-Star** for chemiluminescent visualization of alkaline phosphatase-conjugated antibodies (Signal recorded on X-ray film)
- ▶ **BM Purple** for chromogenic visualization of alkaline phosphatase-conjugated antibodies (Signal recorded on the membrane)

### Critical factors for successful Western blots

In a Western blotting procedure, many variables affect the outcome. We have listed below a few controllable factors that, in our experience, most affect the success of the Western blot procedure. Use these as guidelines for designing your own Western.

**Note:** For general information on the Western blotting procedure, see Towbin and Gordon (1984).

### Preparation of a sample containing a tagged protein

The most common, convenient way to obtain a protein sample for a Western blot is to resuspend and lyse cells directly in an electrophoresis sample buffer (as in Procedure I later in this section), then remove insoluble cell debris by centrifugation. The clarified sample can be loaded directly on an acrylamide gel.

Alternative sources of Western blot samples (which may be mixed with electrophoresis sample buffer) include:

- ▶ Cell extracts
- ▶ Fractions from chromatography column eluates
- ▶ Immunoprecipitates

**Note:** Buffers for these samples must be compatible with the electrophoresis sample buffer. For instance, in the samples, avoid using buffers containing potassium salts since potassium will form a precipitate with the SDS in the electrophoresis sample buffer.