

Labeling Strategies

There are two labeling methods, direct and indirect. **Direct labeling** (also known as **primary labeling**) labels a **primary molecule of interest**. **Indirect labeling** uses a **secondary label** to detect the molecule of interest.

Direct Labeling

Direct labeling (**Figure 1A**) reduces experiment times and can reduce non specific binding. There are a variety of labeling kits available through Life Technologies' website (search "Labeling Kits"). Zenon dye, for example, directly labels the Fc portion of an antibody. The disadvantage to using direct labeling is that any modification to the target molecule can affect binding characteristics. Also, it can be more expensive and difficult to make if commercial kits are unavailable.

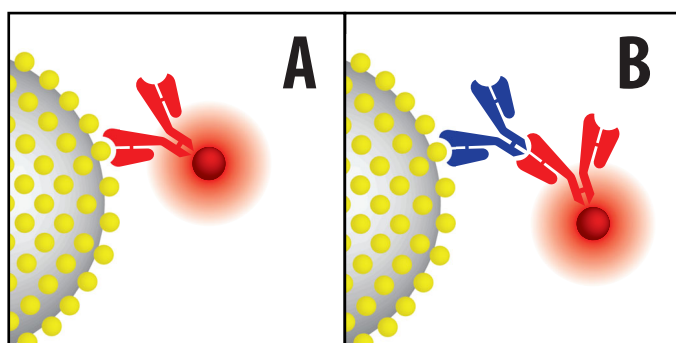


Figure 1. (A) Direct Labeling. (B) Indirect Labeling.

Indirect Labeling

Indirect labeling (**Figure 1B**) is the most common labeling strategy. There are a wide variety of commercially available secondary antibodies that are fairly inexpensive. We have had good results with labels from Jackson Labs (jacksonimmuno.com). See **Table 1** for more strategies.

If the target molecule is not an antibody and lacks a tag, a complimentary antibody can be used. The complimentary antibody can be directly labeled (**Figure 2A**) or indirectly labeled (**Figure 2B**).

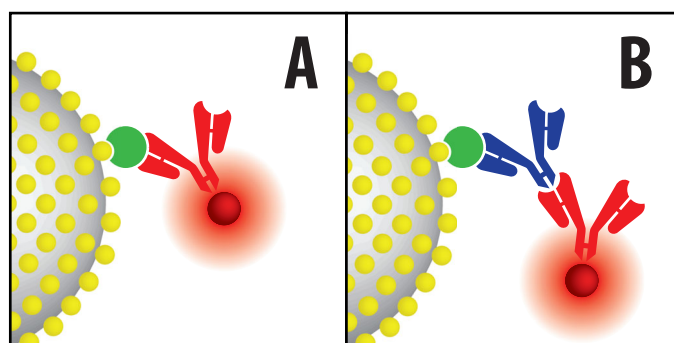


Figure 2. (A) Directly labeled complimentary antibody. (B) Indirectly labeled complimentary antibody.

Anti-species (H+L)*	Antibodies that bind to multiple epitopes on an antibody.
Anti-species Fc specific*	Antibodies that bind to the Fc portion (tail) of the antibody.
Anti-species subclass specific*	Antibodies that bind to a specific subclass of IgG (e.g. 1, 2a, 2b, 3).
Anti-species Fab or (Fab)₂ specific*	Antibodies that bind to a Fab or (Fab) ₂ fragment.
Anti-Tag Antibodies	Antibodies that bind to a variety of tags on target molecules (e.g. Biotin, Flag, HIS-tags, etc).
Labeled Streptavidin	Protein that binds to biotinylated molecules.

Table 1. Indirect Labeling Strategies.

* Can also be purchased as Minimum Cross which means they have been adsorbed against other species to limit cross reactivity.