Introduction
An important key to a successful Western blot is often overlooked – selection of the blocking buffer. In the Western blotting workflow, blocking is done after proteins are transferred from the gel to the membrane, and before the membrane is incubated with the primary antibody (Figure 1). The purpose of the blocking step is to prevent or reduce nonspecific binding of the primary and secondary antibodies to proteins on the membrane or to the membrane itself.

When blocking works optimally, background is eliminated and the signal-to-noise ratio is increased, providing cleaner blots and more robust data. Researchers may spend a great deal of time optimizing many aspects of their experiment – identifying the optimal amount of sample to load on the gel, the buffer makeup and acrylamide percentage of the gel, the type and pore size of the membrane, and the dilution factor for primary and secondary antibodies – but use a standard blocking buffer without considering the potential effects of different blocking agents and blocking buffer components on their experimental system. The dramatic effect blocking buffer can have on Western blot data is demonstrated in Figure 2.

Blocking buffer components
Blocking buffer may be used in two places in the Western blotting workflow. First, the membrane is incubated with the blocking buffer during the blocking step. Additionally, the primary and secondary antibodies may be diluted in the same or an additional blocking buffer during the antibody incubation steps to further prevent nonspecific binding. When designing a blocking buffer, a balance must be reached between preventing nonspecific binding while not disrupting the specific antibody-antigen binding, and components must be chosen that will not interfere with downstream detection systems. Though finding the best blocking buffer may require a process of trial and error, there are some characteristics of blocking buffer components that can help guide selection of the initial blocking buffer tried.

Blocking agents
The blocking agent is the blocking buffer component that will bind to or "block" nonspecific binding sites that would otherwise adsorb primary or secondary antibody. The blocking agent may be protein or non-protein based. Some of the more commonly used blocking agents are discussed below.
**Blocking agent**  | **Considerations**  
---|---  
Nonfat dry milk  | • Inexpensive  
| • Along with BSA, one of the most frequently used blocking agents  
| • Contains phosphoproteins; may interfere with some antigens  
| • Contains avidin; may interfere with some detection systems  
BSA  | • Along with nonfat dry milk, one of the most frequently used blocking agents  
| • Compatible with most antigens and detection systems  
Casein  | • Is a phosphoprotein so may interfere with some antigens  
Fish gelatin  | • Does not contain mammalian proteins; may reduce potential for cross-reaction of blocker components with mammalian antibodies  
PVP  | • Does not contain protein; may reduce potential for cross-reaction of blocker components with antibodies  
Tween-20  | • Does not contain protein; may reduce potential of cross-reaction of blocker components with antibodies  
| • Predicted to disrupt weak interactions but not stronger, specific antibody-antigen interactions  
| • May cause loss of proteins from membrane
**Other considerations**—Blocking buffers are often prepared in phosphate-buffered saline (PBS) or Tris-buffered saline (TBS). For detecting phosphorylated proteins, TBS is recommended because PBS may interfere with the antibody-antigen interaction. PBS can also interfere with alkaline phosphatase detection systems.

Finally, good laboratory technique is important to achieving optimal blocking. Blocking buffers should be freshly prepared and free of particulates. During incubation in blocking buffer, the blot should be agitated to allow even distribution of the blocking agent over the surface of the blot and to prevent settling of particulates onto the blot. And, blocking time should be controlled; in some blocking buffers, long blocking times can result in loss of protein from the blot (DenHollander 1989).

**Take the guessing out of choosing a blocking buffer**

Azure Biosystems offers three blocking buffers to simplify Western blot optimization. Azure Chemi Blot Blocking Buffer is formulated to reduce background and increase signal with chemiluminescent blots. Azure Fluorescent Blot Blocking Buffer provides effective blocking while also stabilizing fluorescent signal. Azure Protein-Free Blot Blocking Buffer is protein-free to reduce background with antibodies that demonstrate a high degree of cross-reactivity with protein blocking agents such as nonfat dry milk, BSA or casein (Figure 2). Contact Azure for more information about which blocking buffer is best for your experiment.

**References**