Western Transfer FAQ

1. How long will the immunostaining take when PeproTech's Western Transfer protocol is followed?
   The Western Transfer process will take approximately 6 hours from transfer of proteins to visualization of bands.

2. What type of molecular weight marker should be used?
   PeproTech uses the Novex® Sharp Pre-stained Protein Standard in all Western Transfers performed in-house, although this molecular weight marker does not have to be utilized. However, it is necessary that a pre-stained molecular weight marker is used when not following the ECL detection method.

3. Is the addition of a positive control necessary in a Western Transfer?
   Yes, in order to know exactly how the protein of interest will visualize on the Western Transfer you must add a positive control to your gel. When using PeproTech's antibodies in a Western Transfer, we recommend using the corresponding PeproTech recombinant protein, which was the immunizing antigen for the antibody of choice.

4. Is agitation of the membrane essential during the incubation periods?
   Yes, it is essential that the membrane be agitated during the incubation periods. If the membrane is not agitated the antibodies, blocking buffer, and washing buffer may not affect the membrane evenly and can create a splotchy or patchy background. It can also limit the detection of the proteins by the antibodies.

5. Is it necessary to include the blotting paper during the protein transfer step?
   Yes, it is necessary to include the blotting paper as a barrier in the protein transfer step, as it helps protect the gel and mem brane from any possible damage resulting from direct contact with the sponges, yet does not interfere with the electric current.

6. Does the color development system recommended in PeproTech's Western Transfer protocol have to be used?
   No. There are many different color development systems that can be used for the visualization of your Western Transfer. However, the system that is chosen must be compatible with the enzyme conjugate being used. PeproTech uses an alkaline phosphatase linked secondary antibody. NBT/BCIP is suitable for use with this enzyme, and is therefore used for the visualization of our Western Transfers.

7. Why are some Western Transfer results stronger than others when using different lots of the same antibody?
   Due to the nature of polyclonal antibodies, variability may be seen from lot to lot.

8. Can an enzyme-conjugated primary antibody be used in a Western Transfer rather than using a primary/secondary antibody system?
   Yes, an enzyme-conjugated primary antibody can be used instead of utilizing a primary/secondary antibody system in your Western Transfer. However, by using a labeled secondary antibody that recognizes the antigen-specific primary antibody, there will be an amplification of the signal seen in a Western Transfer when compared to using the enzyme-conjugated primary antibody alone.