Primary Antibody Detection

Direct and Indirect Detection Methods

The outcome of an IHC-P assay strongly depends on the detection system used to visualize the target antigen. The **direct detection** method, namely labelling the primary antibody with enzymes or fluorochromes, is fast and enables direct detection of the target antigen without the requirement of a secondary antibody. However, as only one tagged antibody binds to each antigen, the signal may be too weak for detection under the microscope if the antigen concentration is low. Therefore, indirect methods are preferred.

In indirect methods, the primary antibody is not labelled. Instead, a labelled secondary antibody which is directed against the host species of the primary antibody is used to amplify the signal, often in combination with additional amplification steps. Common labels are fluorescent conjugates or enzymes which convert soluble substrates to chromogenic precipitates (see Fluorescent-and-chromogenic-IHC-P).

SYSY's reference protocols mostly use the **Avidin-Biotin Complex (ABC)** method for signal amplification and chromogenic substrates for antigen-antibody-complex detection. In the ABC method, biotin conjugated secondary antibodies link primary tissue-bond antibodies with avidin-biotin-enzyme complexes. **The LSAB method is very similar to the ABC method and uses streptavidin-peroxidase complexes instead of avidin** . Certain tissues (e.g. kidney, liver) contain high amounts of endogenous biotin. Particularly when using Tris-EDTA or EDTA-based antigen retrieval buffers, the ABC method can lead to high background and / or false positive staining. SYSY thus recommends using an avidin-biotin blocking step. If the background problem persists, consider trying a polymer-based detection system instead of the ABC method.

In **polymer-based detection systems**, enzymes and secondary antibodies are conjugated through polymer backbones. The large number of conjugated antibodies and enzymes enhance sensitivity compared to the ABC method.

The **Tyramide Signal Amplification (TSA)** methodology is an enzyme-mediated detection method. It uses horseradish peroxidase (HRP) to catalyze the deposition and binding of labeled tyramide (e.g. biotin, DNP) onto tissue sections. The labels can then be detected using chromogenic or fluorescent detection methods. TSA increases the sensitivity up to 100-fold compared to the ABC method, thus enabling the detection of low abundance proteins.

Mass Spectrometry ImmunoHistoChemistry (MSIHC) is a very new approach. It employes antibodies that are labeled with metal isotopes of known molecular mass followed by MS imaging.

Pitfalls when using Secondary Antibodies

Many indirect detection methods are based on the use of secondary antibodies. However, secondary antibodies may cross react with endogenous immunoglobulins in the tissue. This can be minimized by using cross-adsorbed secondary antibodies and the appropriate blocking solutions. When using normal serum for blocking, choose normal serum from the host species of the secondary antibody.

SYSY recommends including a 'no primary antibody control' in your IHC-P staining experiment. Omitting the primary antibody in the staining protocol ensures that staining is produced from primary antibody binding to its antigen and not by the detection system or the tissue specimen.