Antibody Selection Guide

Complexities

Many commercial antibodies and in vitro diagnostic tests only demonstrate their advertised properties in the context of a specific tissue or assay platform. Some well-recognized complexities of IHC using FFPE include:

- Abs can show specific interactions with the mature protein in blots or other assays, but still lack sensitivity or specificity when applied to complex tissues;
- Abs that show a single reactive species in blots of one complex tissue can show unrelated cross-reacting species in another.
- Specificity can differ between fixed and unfixed tissues because of unpredictable effects of fixation and embedding on specific and nonspecific Ab binding.
- Even if an Ab shows the expected pattern of reaction in assays using the recommended positive control, different patterns of reactivity can be observed in the context of other tissues or disease states.

Criteria for antibody selection

The first step in Ab selection is to carefully compare the manufacturer's data sheets and associated primary literature for the competing products. There is no gold standard approach. However, the collected information can help identify the most suitable reagents. Although a systematic comparison of reagents takes time, it is almost always time very well spent.

- Preference should be given to Abs known to specifically react with the protein in FFPE tissue. If the manufacturer does not indicate suitability for IHC, there is probably a reason.
- Mouse or rabbit monoclonal Abs (MoAb) or rabbit polyclonals are preferred for the Ventana platform. Other primary Abs may require manual assays or additional optimization.
- MoAbs are often preferred over polyclonals, but can have their own limitations. MoAbs recognize a specific epitope, but this does not mean that they are protein specific. In addition, some MoAbs possess charge or hydrophobic properties that result in remarkably specific appearing non-specific reactions.
- Because MoAbs recognize a single epitope, they are sometimes less sensitive than a polyclonal Ab, and may not be usable for fixation-sensitive epitopes.
- Preference should be given to highly purified Igs or affinity-purified Abs, which can then be mixed with purified blocking proteins or other defined reagents. Whole serum, crude serum fractions, ascites, or unfractionated culture supernatants often show nonspecific reactions, particularly with low titer antibodies. For example, natural or autoantibodies in antisera can lead to nonspecific staining.
- Antigen purity is often critical for polyclonal Abs. Although specificity can be enhanced by affinity purification, effectiveness depends on the purity of the antigen coupled to the affinity column. Many affinity-purified polyclonal Abs are not specific for the putative antigen.
- Most commercial MoAbs are IgG1 and IgG2 and the core has appropriate control Igs. Studies with other isotypes will often require the investigator to obtain the corresponding isotype control.
- Preference should be given to high titer Abs. Low titer Abs are more often associated with background problems. They also increase reagent costs and can preclude migration of the assay to a high-throughput automated platform. Ventana dispensers are designed to accommodate relatively large volumes and there is a significant hold back volume (100 microliters) that limits complete utilization of the reagent.
- If a synthetic polypeptide was used as antigen, it is important to critically examine the sequence to avoid confounding specific cross-reactions. Have homology searches been performed against available human databases? Is the parent protein a member of a known protein family and/or is the sequence homologous

to sequences found in unrelated proteins? Preliminary homology searches (e.g., with BLASTP) are recommended for all Abs using peptide antigens.

- When possible, studies of human tissues should employ Abs to human antigens or sequences. Specificity in one species does not insure specificity in another. Even highly homologous antigens can show significant differences in interspecies cross-reactivity.
- When possible, preference should be given to MoAbs with known epitopes. It can be helpful to know whether the epitope is sequential or conformational; whether reactivity is dependent on disulfide bonds, divalent cations or other factors; and the predicted location of the epitope on the native protein. Sequential epitopes may be more readily detected in FFPE. On the other hand, a buried sequential epitope that requires denaturation or sulfhydryl reduction for detection by Western blot may be masked in the tissue.