Antibody Validation: How Do We Determine Specificity?

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Abstract

Antibody specificity reflects the ability of an antibody to distinguish between two different epitopes. Their precise ability to discriminate between overlapping epitopes can be determined by using appropriate control antigens. The National Cancer Institute (NCI) Anti-Body Screening and Validation Program is a joint effort by NCI and the Kaleidoscope Group that is supporting the development and validation of antibodies for use in research and diagnostic applications. Several factors, including antigen specificity, affinity, and cross-reactivity, need to be considered to ensure that an antibody is specific for the intended target. This poster will outline our approach to antibody validation and highlight the antibody validation process that we have taken on for our research platform.

Methods

For each of the targets of interest, commercial antibodies were chosen based on testing on tissue with IHC, literature citations, species reactivity, peptide availability, and vendor reliability. For each of these targets of interest, commercial antibodies were chosen based on testing on tissue with IHC, literature citations, species reactivity, peptide availability, and vendor reliability. In each case, controls were set up to confirm the specificity of the antibody. The process of antibody validation is complex, and specificity testing is just one aspect of the process. Here we’ve outlined our approach for identifying the best commercial antibody to use for our research studies. Using both qualitative and quantitative approaches, we have identified which antibodies that we have chosen on antibody based on multiple criteria. Literature searches are important for identifying how an antibody may stain between varying epitopes can and does vary from one supplier to the next. With new molecular targets being identified for improved clinical outcomes, we must be confident in the antibodies we choose to use in our research.

Results

This table summarizes the qualitative and quantitative antibody validation criteria.

Discussion

The process of antibody validation is complex, and specificity testing is just one aspect of the process. Here we’ve outlined our approach for identifying the best commercial antibody to use for our research studies. Using both qualitative and quantitative approaches, we have identified which antibodies that we have chosen on antibody based on multiple criteria. Literature searches are important for identifying how an antibody may stain between varying epitopes can and does vary from one supplier to the next. With new molecular targets being identified for improved clinical outcomes, we must be confident in the antibodies we choose to use in our research.

References


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