Optimized Human on Human Immunodetection

Introduction

Human or humanized antibodies are increasingly being used to treat cancer, autoimmune disease, and other disease conditions. A key step in therapeutic antibody development is confirming that the antibody binds to the intended target in human tissues and not to other targets or in unexpected tissues. The US Food and Drug Administration (FDA) recommends that antibody drug candidates undergo tissue cross-reactivity (TCR) tests against 42 human tissues to confirm on-target binding and check for off-target binding that could trigger adverse effects (1, 2).

Immunohistochemistry (IHC), where a primary antibody bound to test tissue is detected with a color reaction, is a preferred technology for TCR testing. Since most therapeutic antibodies are either fully human or humanized, standard IHC methods that incorporate sequential incubations with an unconjugated primary antibody followed by an anti-human secondary antibody are impractical due to high concentrations of endogenous human antibodies. In this situation, a secondary antibody directed against human IgG cannot distinguish between the therapeutic antibody and endogenous IgG, resulting in background staining. Other tests must then be used to distinguish this background from true off-target binding. Background staining can be reduced by lowering the amount of primary antibody or shortening incubation times, but high assay sensitivity is essential to avoid missing real off-target effects.

Currently, the leading IHC methodology used to circumvent background staining when detecting human primary antibodies on human tissue sections involves conjugating the primary antibody with a hapten tag, usually biotin or fluorescein isothiocyanate (FITC). Incorporating these tags enables detection of the primary antibody through the hapten, rather than through an anti-human IgG secondary antibody. Hapten conjugation to the primary antibody, though standard practice, has significant shortcomings. In general, any modification of the candidate compound (in this case, an antibody) raises concerns that the properties of the prospective drug may have changed. A specific concern is that the labeling procedure has altered the target binding profile of the primary antibody, but any uncertainty around the properties of the candidate should be avoided. In addition to the risk of potential effects on the test material, significant time and expertise will be required to perform the conjugation and subsequent validation of the labeled primary antibody.

A second method of potentially detecting human antibodies on human tissue sections involves pre-complexing the human antibody with an anti-human IgG secondary antibody, then using human serum to block unbound secondary antibody [3]. This method suffers from the complexity of optimizing the ratio of human antibody to secondary antibody, and the difficulty of effectively eliminating the background.

Here, we report on a new product from Vector Laboratories; the H.O.H.™ (Human on Human) Immunodetection Kit (HOH-3000), that simplifies the IHC procedure, greatly reduces assay time by eliminating the practice of conjugating the primary antibody and achieves high sensitivity of target antigens while excluding background originating from endogenous IgG. We compared staining results of this kit against the hapten-tagged antibody method using several human (or humanized) antibodies on a range of human tissue specimens. Additional benchmarking data is also presented, comparing the H.O.H. Kit against the primary antibody pre-complex approach.

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