

# The Advantages of Using Immunofluorescence (IF) to Screen Humanized Antibodies as Potential Therapeutic Candidates

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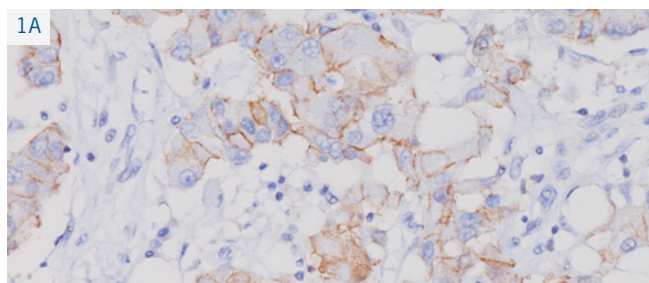
With the rise of therapeutic human monoclonal antibodies, immunohistochemistry (IHC) assays have been developed to screen and detect human antigens on human tissues.<sup>1</sup> While brightfield assay quantification is not always easy, immunofluorescence (IF) assay use is rapidly growing because quantifiable data using specialized imaging software is readily available. A human-on-human assay utilizing IF is advantageous for effective screening of these antibodies on human tissue, providing valuable insight as to an antibody's potential as a therapeutic treatment.<sup>2</sup>

While Biocare Medical's Human-on-Human HRP Polymer kit was originally intended to be visualized through use of a chromogen, using a fluorophore can be a simple substitution. For either method, the humanized antibody needs to first be labeled with digoxigenin. After the labeled primary antibody has incubated on the tissue, a mouse anti-digoxigenin secondary is applied. Following its incubation, MACH 2 Mouse HRP-Polymer (supplied in the kit) and a chromogen are applied to the IHC slides, whereas an anti-mouse fluorescent conjugate is used for IF visualization. The tissue is then counterstained by hematoxylin for IHC or DAPI for IF and mounted accordingly.

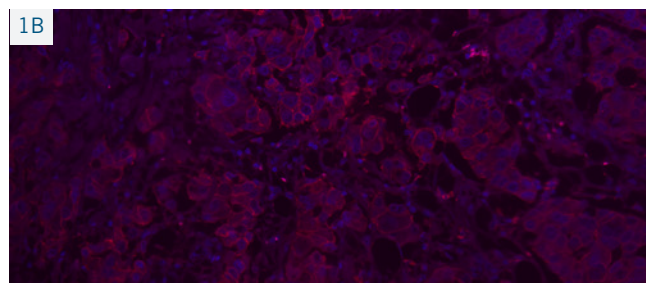
To exemplify this substitution, human monoclonal HER2, CD6, and Histone antibodies were detected through IHC and IF utilizing Biocare's Human-on-Human kit. After comparing results from both methods, identical staining patterns were observed while the IF staining yielded precise, quantifiable results with little to no background.<sup>3</sup>

Human-on-human immunofluorescence assays provide a reliable and simple method to detect and quantify humanized antibodies on human tissue, proving especially useful when researching potential therapeutic treatments.

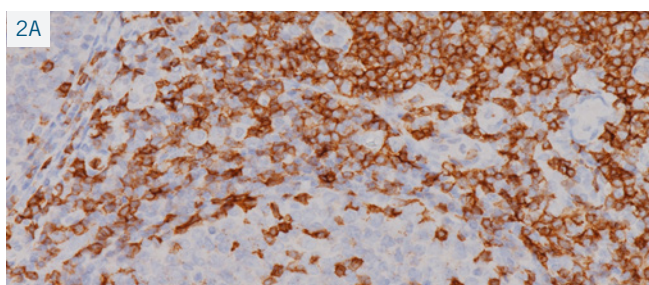
## Figures 1-3



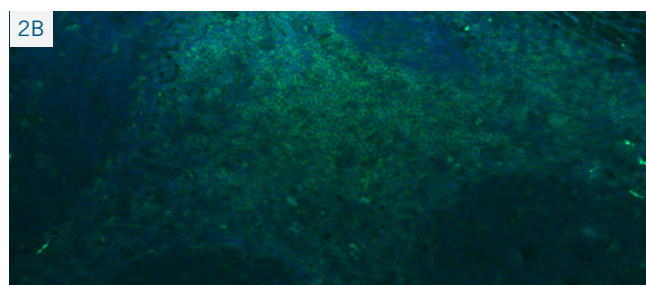
Breast cancer stained with Human HER2 [proprietary] with DAB using the IHC HH assay



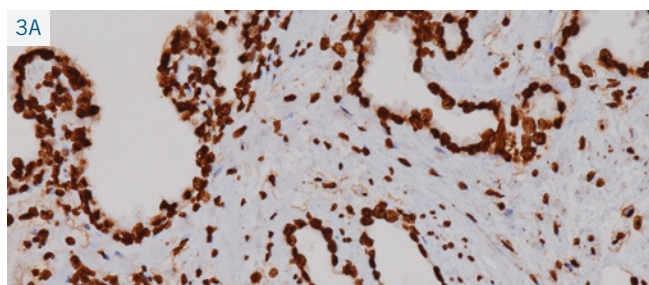
Breast cancer stained with HER2 [proprietary] detected using CF@594 IF HH assay



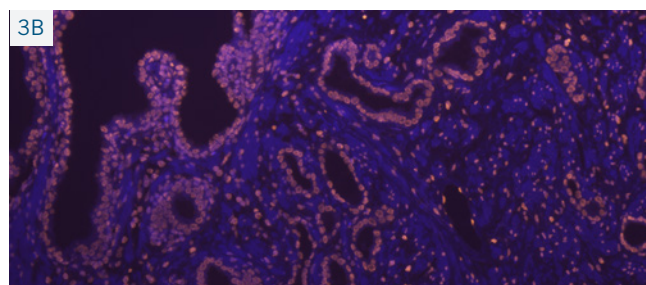
Tonsil stained with CD6 [3F7B5] with DAB using the IHC HH assay



Tonsil stained with CD6 [3F7B5] detected using CF@488 IF HH assay



Prostate cancer stained with Histone H2B [IGX4228H] with DAB using the IHC HH assay



Prostate cancer stained with Histone H2B [IGX4228H] detected using CF@543 IF HH assay

To learn more about the Human-on-Human HRP Polymer, please contact us anytime at 800-799-9499 or check out the kit here: <https://biocare.net/product/human-human-hrp-polymer/>.

[www.biocare.net](https://www.biocare.net)

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1. Chames, P, *et al*. Therapeutic antibodies successes, limitations and hopes for the future. *Br J Pharmacol*. 2009; 157(2):220-33

2. Figueroa S., *et al*. The development of an immunofluorescence assay in the detection of human antibodies in human tissues. 44th Annual NSH Symposium/Convention. National Society of Histology. America's Center Convention Complex, St. Louis, MO. 20 Sept. 2018. Poster Presentation

3. Vargas J., *et al*. A simple and rapid method for the detection of human and/or humanized antibodies on human tissues by immunohistochemistry. 43rd Annual NSH Symposium/Convention. National Society of Histology. Gaylord Palms Resort & Convention Center, Kissimmee, MO. 17 Sept. 2018. Poster Presentation