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.

Study Overview

As mentioned, the predominant methodology currently in use for detecting human primary antibodies on human tissue sections for therapeutic antibody assessment is the hapten-tagged antibody approach. Our primary focus in this study was to compare workflows and staining results on frozen tissue sections between the hapten-tagged method and the Vector Laboratories' H.O.H. Kit method. Therefore within this study overview, we provide a brief outline of the main reagents used to conduct the IHC assays comparisons. Unless otherwise noted, all reagents used were from Vector Laboratories.

Tissue Preparation

Cryopreserved and formalin-fixed, paraffin-embedded (FFPE) tissue blocks were obtained from the Cooperative Human Tissue Network at Vanderbilt University (see Table 1).

Table 1. Human tissue specimens evaluated*

Human Tissue Specimens Evaluated for Cross-Reactivity with the H.O.H. Kit		
	FFPE	Frozen
Normal	Tonsil	Tonsil
	Lymph Node	Lymph Node
	Endometrium	Uterus
	Prostate	Prostate
	Thymus	
	Kidney	
	Spleen	
	Skin	
	Placenta	
	Testis	
	Skeletal Muscle	
	Thyroid	
	Brain	
Abnormal	Breast Cancer	Breast Cancer
	Lymphoma	
	Mesothelioma	
	Melanoma	

**Most of these tissues, particularly FFPE material, were used as negative controls only to determine absence of staining during product development of the H.O.H. Kit. The intent was to gauge cross-reactivity of the detection reagents with tissue components from a variety of human organ specimens.*

Frozen tissue sections were cut (5 µm), air dried, and fixed with acetone for 5 minutes. Fixed frozen sections were then stored short term at -80°C until needed. FFPE tissue sections were cut (5 µm), deparaffinized in xylene, rehydrated through graded alcohol, and then subjected to standard antigen retrieval treatment in a pressure cooker with Vector Laboratories' Antigen Unmasking Solution, Tris-Based (H-3301).

Antibodies

A panel of unconjugated human, humanized, and chimeric primary antibodies was obtained from commercial vendors (see Table 2). Further information regarding these primary antibodies is available on the respective vendor's website. For simplicity in this paper, we refer to all the various formats of the antibodies listed in Table 2 only as human primary antibodies.

Unconjugated human primary antibodies were used in conjunction with the H.O.H. Kit. No additional purification, concentration, or similar means of modifying the supplied antibodies was performed when using them with the H.O.H. Kit. Aliquots were taken directly from the supplied stock material and used according to the kit instructions.

To compare the H.O.H. Kit with the hapten-tagged antibody methodology, clinically significant primary antibodies PD-1, and HER-2, were labeled at Vector Laboratories with FITC to ~2-3 fluorophores per IgG molecule. The decision to use FITC instead of biotin as the antibody tag was made to avoid potential issues with detectable levels of endogenous biotin in some tissues.

Table 2. Human primary antibodies used in this study

Antibody	Type/Isotype	Source	Cat. No.
PD-1	Human IgG4	BioVision, Milpitas, CA, USA	A-1306
HER-2	Human IgG1 κ	BioVision, Milpitas, CA, USA	A-1046
CD20	Human IgG1	BioVision, Milpitas, CA, USA	A-1049
CD52	Human IgG1 κ	Absolute Antibody, Boston, MA, USA	Ab00705
CD4	Primatized IgG4 λ	Absolute Antibody, Boston, MA, USA	Ab00450
Desmin	Human bivalent Fab	Bio-Rad Laboratories, Hercules, CA, USA	AbD03744
Cytokeratin	Human bivalent Fab	Bio-Rad Laboratories, Hercules, CA, USA	AbD03748

IHC Procedures

Table 3 provides a comparative overview of the required steps to achieve IHC staining with the H.O.H. Kit and FITC-tagged primary antibody methodologies.

The step-by-step instructions (User Guide) for the H.O.H. Kit provided in Appendix 1 are also available for download from the Vector Laboratories' website (vectorlabs.com/HOH).

The times indicated in Table 3 for conjugation, clean-up, and subsequent validation of the primary antibodies in the FITC-tagged antibody method were obtained from protocols by, and discussions with, lab personnel who routinely use this methodology. Note that reliance on third-party or interdepartmental expertise to perform conjugation and validation steps of the primary antibodies is a common practice to enable IHC. We acknowledge the variability that can occur when conducting these steps in different companies, organizations, and research institutes. However, while the conjugation and validation steps could be performed faster than indicated, the critical point is that these steps are simply not required with the H.O.H. Kit, thereby saving significant time and possible reliance on outside sources.

Table 3. IHC Workflows comparing H.O.H. Kit and FITC-Tagged Primary Antibodies

Steps required for IHC	H.O.H. Kit	FITC-tagged primary antibody
Conjugate primary antibody and perform clean up steps	N/A	1-5 days
Validate conjugated primary antibody	N/A	1-5 days
Prepare primary antibody	~ 1 Hour	~ 10 minutes
Block tissue sections	~ 20-40 minutes	~ 20-40 minutes
Apply primary antibody to tissue sections	30-60 minutes	30-60 minutes
Detect with HRP conjugate*	30 minutes	30 minutes
Apply DAB substrate	~ 5-10 minutes	~ 5-10 minutes
Total time to prepare primary antibody and conduct IHC assay	**< 3 hours**	**2 days to > 1 week**

N/A: Not Applicable
**HRP anti-goat IgG is a component in the H.O.H. Kit. HRP anti-FITC was used to detect the FITC-tagged antibodies.*

IHC Workflow notes:

1. Standard buffer washes (2-3 x 5 minutes) were conducted between each incubation step.
2. All incubations and buffer washes were performed at room temperature (~20-23 °C).
3. Some labs incorporate overnight incubation of the primary antibody at 4 °C. We did not find that necessary in this study. If overnight incubation is part of an established lab SOP using the hapten tagged antibody approach, it would increase the total time.

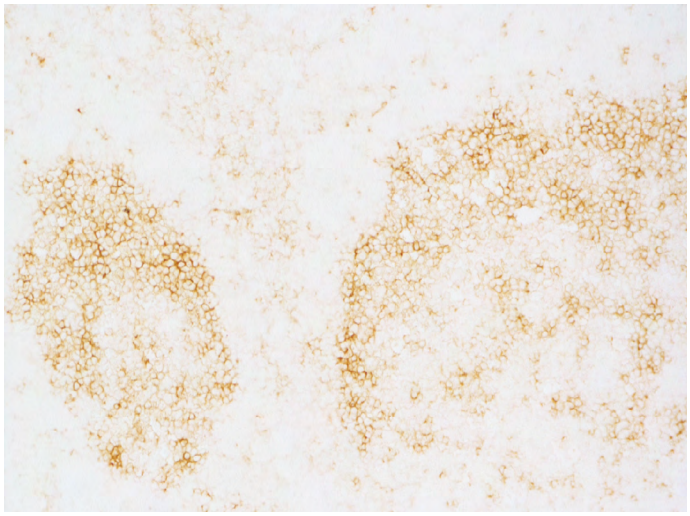
Results

After conjugating the PD-1 and HER-2 antibodies with FITC to conduct the comparison study using the IHC workflows outlined in Table 3, the following staining results were obtained on serial sections of the tissues indicated. Since most labs that run IHC assays with human antibodies on human tissue sections use frozen specimens, only frozen sections were used for these method comparison assays.

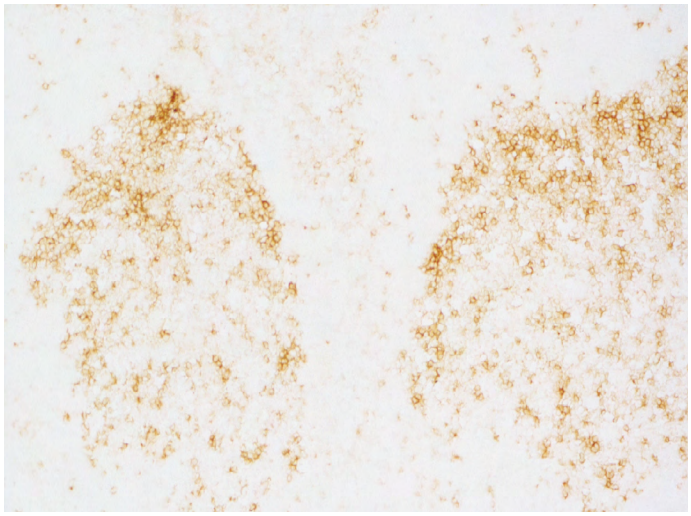
Comparative Antigen Staining on Frozen Sections

Figure 1. PD-1 staining on human tonsil serial sections (frozen). No counterstain.

H.O.H. Kit



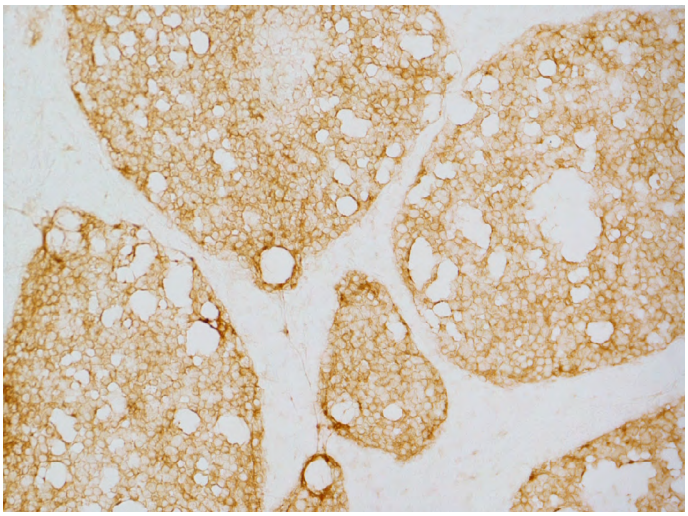
FITC-Tagged Antibody Method



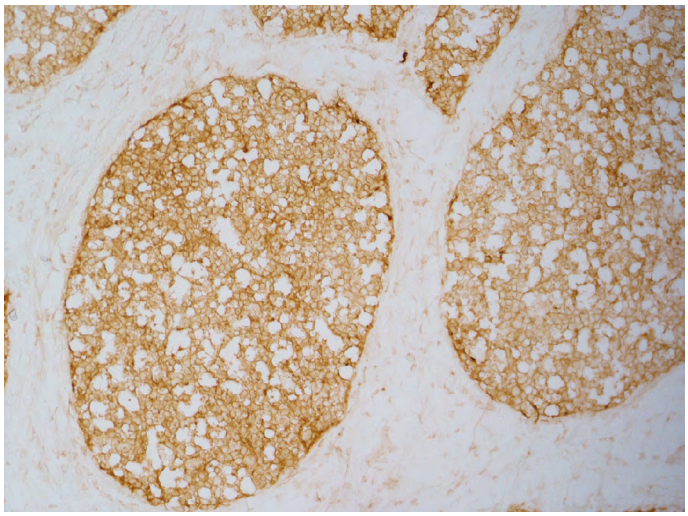
Each staining method demonstrated similar target antigen specificity and sensitivity of PD-1 detection on frozen tonsil tissue sections.

Figure 2. HER2 staining on human breast cancer serial sections (frozen). No counterstain.

H.O.H. Kit



FITC-Tagged Antibody Method



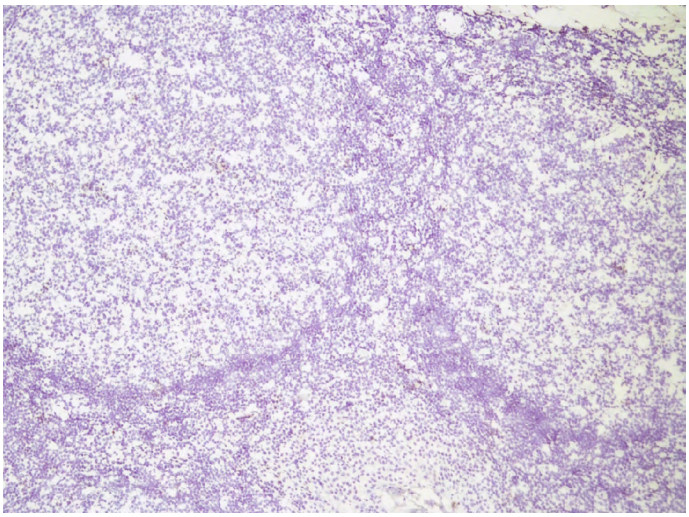
Each staining method demonstrated similar target antigen specificity and sensitivity of HER-2 detection on frozen breast cancer tissue sections.

Negative Control Sections for Frozen Sections

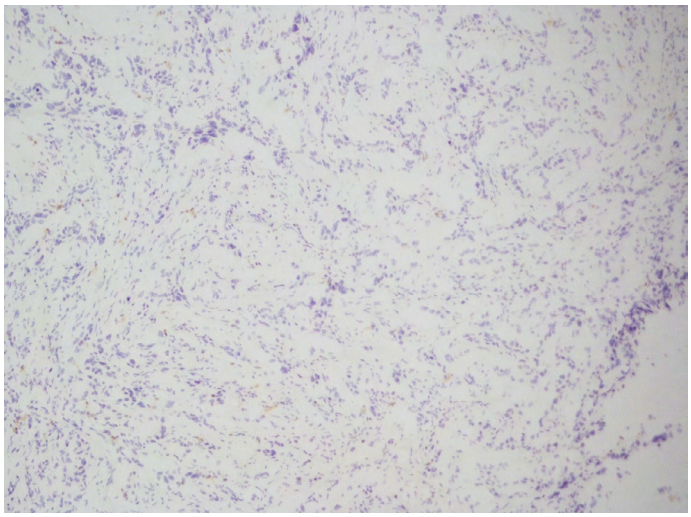
To ensure appropriate target antigen staining was achieved with each method, in each of the tissue specimens in Figures 1 and 2, negative control sections of the same tissues were used.

Figure 3. Human tonsil section (frozen) and human breast cancer section (frozen).

Human Tonsil Section (frozen)



Human Breast Cancer Section (frozen)



The sections shown in Figure 3 were subjected to the H.O.H. Kit procedure; omitting the human primary antibody. A hematoxylin counterstain (blue nuclei) was included to see the underlying tissue morphology since no other staining was present. Negative control sections for the FITC-tagged primary antibodies also showed no staining (results not shown).

The negative control assay data (Figure 3) shows that each method provides background-free staining on the frozen sections. These negative controls directly support the positive staining results shown in Figures 1 and 2 in that each method's specific target antigen localization was achieved with no off-target binding, which by other methods can show potential false-positive staining results.

Further Benchmarking

The comparative study between the H.O.H. Kit and the FITC-tagged primary antibodies did not include performance evaluation on FFPE sections, which are not routinely used in human on human IHC applications to assess therapeutic antibody candidates. However, in research projects that involve working with human primary antibodies, archived human FFPE material may be required due to the scarcity of frozen material and/or when there is a preference for the superior cellular architecture and morphology of FFPE specimens. Additionally, research projects typically have more flexibility to conduct experiments than clinically focused projects, which provides research investigators latitude to evaluate other approaches and capitalize on published methods.

One published method for the detection of primary antibodies raised in the same species as the tissue sections describes a pre-complex approach whereby the primary antibody is pre-bound in solution with a secondary antibody prior to incubation on the tissue section [3]. Residual unbound secondary antibody is absorbed with normal serum from the same species to prevent binding to immunoglobulin present in the sections. This approach is similar to the methodology used in the H.O.H. Kit. Both methods, for example, use unconjugated primary antibodies. However, differences are apparent in the detection methodologies and the means of reducing background staining.

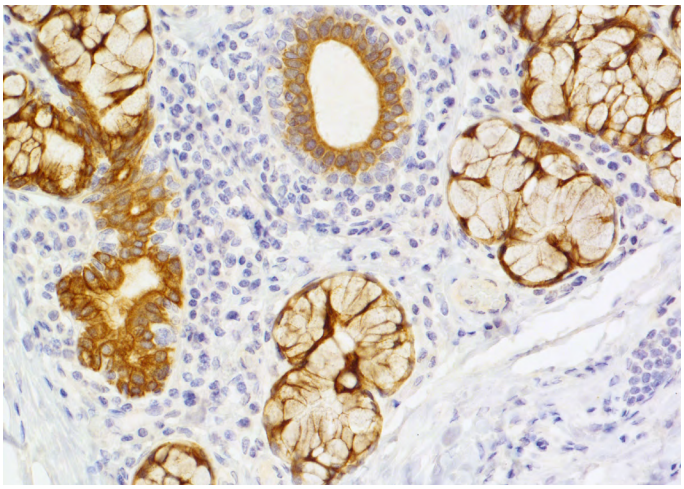
We compared the H.O.H. Kit with the Antibody Pre-Complex Method on human FFPE and frozen tissues listed in Table 1, using the unconjugated human antibodies listed in Table 2. Corresponding reagents from Vector Laboratories were incorporated into the pre-complex workflow. Briefly, unconjugated primary antibodies were incubated with unconjugated goat anti-human IgG secondary antibody. Following guidelines from reference 3, this solution was mixed with human serum, then applied to the tissue sections. An HRP anti-goat polymer and DAB were used for detection and visualization.

An example of staining results comparing the two methods on FFPE sections is shown in Figure 4. Varying levels of background staining were observed in both normal and abnormal tissues (Figure 5) stained with the antibody pre-complex method. All sections stained using the H.O.H. Kit provided clear, unambiguous staining of the target antigen with essentially no discernable background.

The Antibody Pre-Complex Method requires that the test antibody be complexed with an anti-human IgG secondary antibody, then "quenched" with excess secondary antibody before the mixture is applied to the tissue. It's essential to carefully optimize the ratio of the test antibody to the secondary antibody, and to ensure that enough quenching reagent is added to be effective. In the H.O.H. Kit, we simplify assay development by providing ready-to-use reagents where only the test article concentration needs to be optimized, similar to a standard IHC assay. Our modified pre-complexing method takes advantage of a proprietary quenching reagent to remove the need for precise calculations, while maintaining high sensitivity using a highly-purified, sensitive HRP-labeled antibody. This detection approach works well with a wide range of primary concentrations (see Figure 6) and therapeutic antibody formats, including tethered Fab fragments.

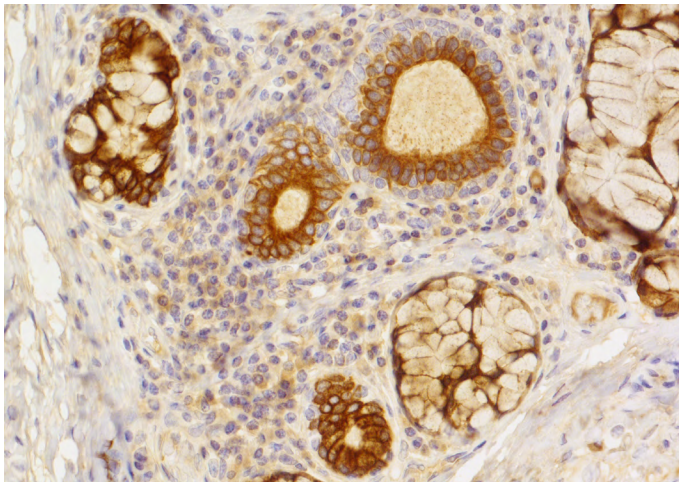
Figure 4. Serial sections of human tonsil (FFPE).

H.O.H. Kit

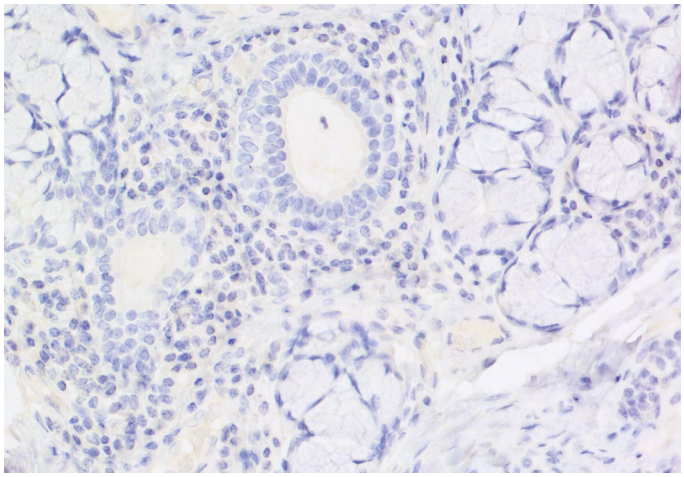


Positive staining (brown) for cytokeratin using the H.O.H. Kit. Note strong specific epithelial staining and no confounding background interference. Hematoxylin counterstain (blue).

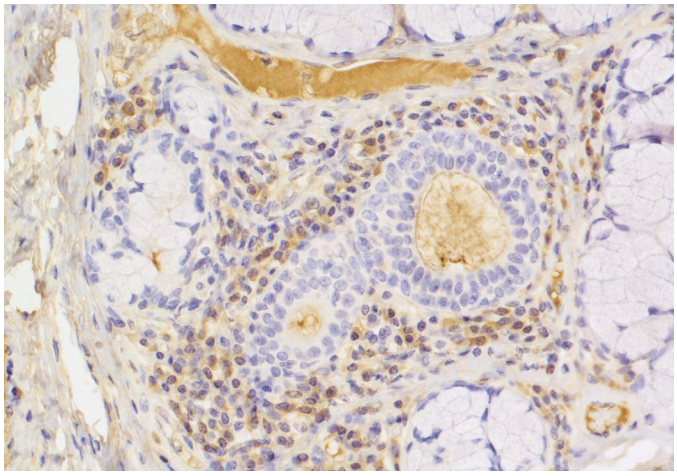
Antibody Pre-Complex Method



Positive staining (brown) for cytokeratin using the Pre-Complex Method. Strong epithelial staining is present along with significant background interference. Hematoxylin counterstain (blue).



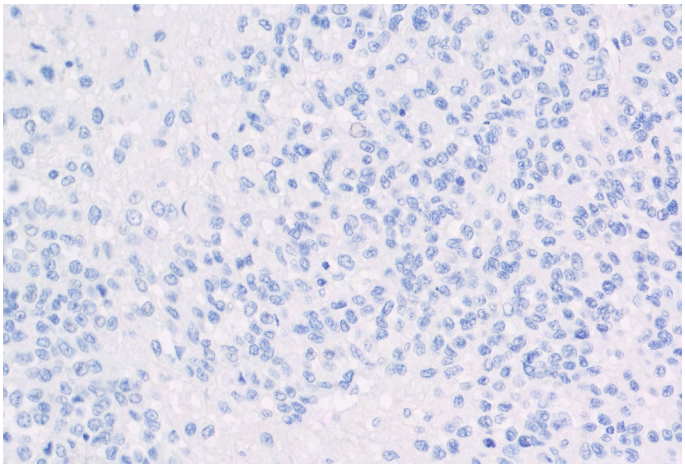
No primary antibody negative control section showing absence of any staining with the H.O.H. Kit. Hematoxylin counterstain (blue).



No primary negative control showing significant background using the Pre-Complex Method. Hematoxylin counterstain (blue).

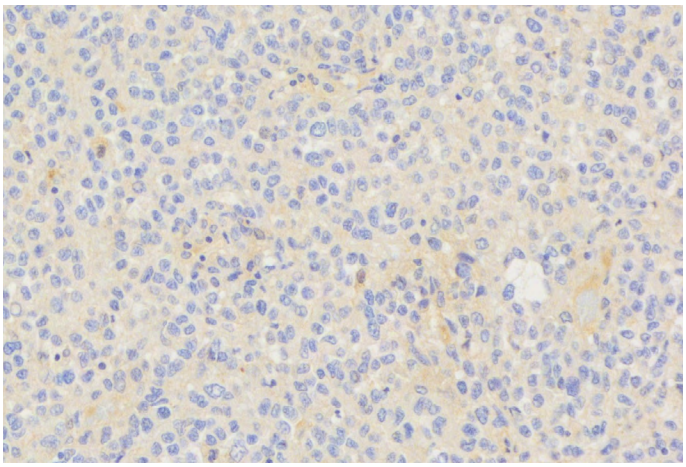
Figure 5. Serial sections of human melanoma (FFPE)

H.O.H. Kit



No primary antibody negative control section showing absence of any staining with the H.O.H. Kit. Hematoxylin counterstain (blue).

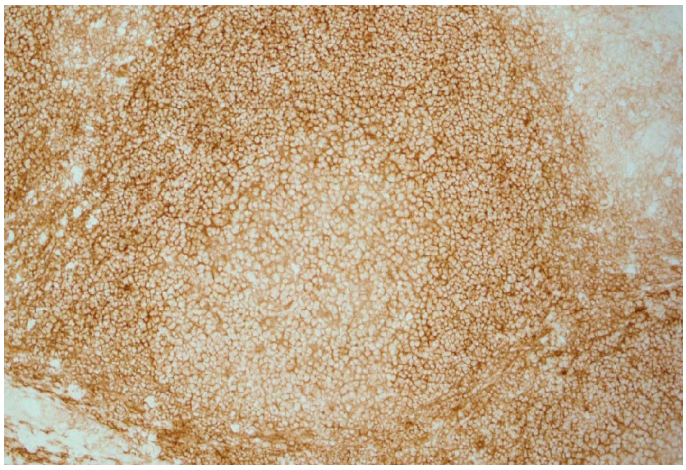
Antibody Pre-Complex Method



No primary negative control showing background using the Pre-Complex Method. Hematoxylin counterstain (blue).

Figure 6. Range of therapeutic antibody concentrations without adjusting the detection reagent concentrations

1:1000



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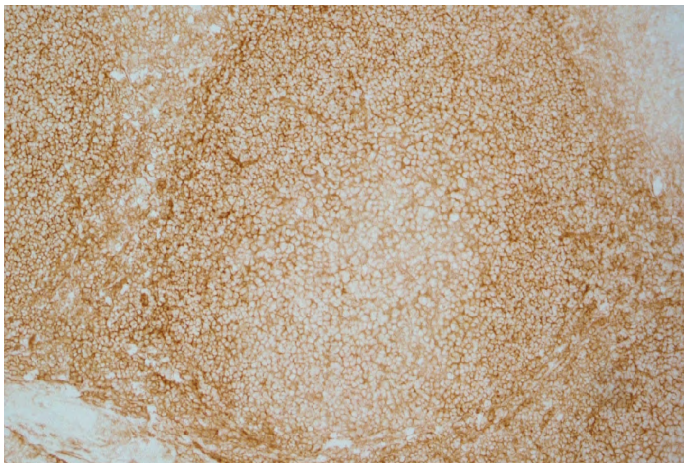
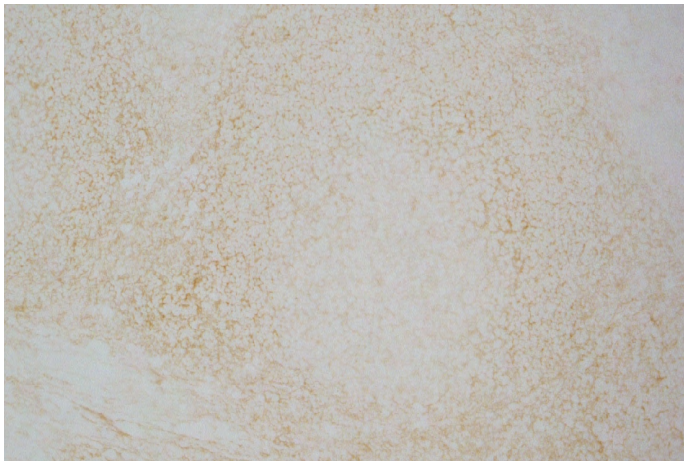


1:4000

1:8000



Serial sections of frozen tonsil were stained with serial dilutions of humanized anti-CD52 and the H.O.H. Immundetection Kit

Discussion

Comparison between H.O.H. Kit and FITC-tagged approach

A key characterization step for candidate therapeutic antibodies involves assessing their cross-reactivity with non-target epitopes in frozen human tissue using IHC. The primary IHC method for this TCR assessment modifies the human primary antibody with a hapten tag, such as biotin or FITC, to avoid the use of an anti-human secondary antibody. A primary disadvantage of this modification of the primary antibody is the increased risk of changing the binding characteristics toward the target antigen. Indeed, other investigators have raised concerns about using FITC-tagged primary antibodies in TCR assessment [4].

In this study, we compared FITC-tagged human primary antibodies with the H.O.H. Kit. As presented in Figures 1, 2, and 3, equivalent staining specificity and sensitivity was achieved for each of the methodologies using defined antibodies on normal and abnormal tissue specimens. Given our results, we did not observe any changes in target specificity between conjugated and unconjugated antibody methods. These results align with typical concerns regarding specificity of hapten-tagged primary antibodies. The antibodies in this study were likely conjugated with an optimal number of FITC molecules that did not affect binding affinity or avidity toward their respective target antigens. An outstanding question is whether this same degree of FITC labeling or labeling with another hapten, would prove as effective on different human primary antibodies that bind different target antigens.

As presented in Table 3, the conjugation and subsequent validation of the tagged primary antibody requires significant time investment before IHC can be started. The times indicated would undoubtedly vary among institutes conducting these studies. Experienced lab personnel who conduct human on human IHC assays routinely have indicated that, the conjugation and validation of primary antibodies can take considerably longer than the times indicated. This is particularly true when interdepartmental resources or external expertise are necessary to modify and validate the antibodies. While we consider the times outlined in Table 3 as a conservative estimate, it is apparent that a considerable bottleneck in conducting TCR studies is the preparation of the primary antibody. Coupled with the time requirements are the additional reagent costs to tag the primary antibody and the significant loss of antibody that occurs during the conjugation and clean-up processes.

Hallmarks of a valid IHC assay are appropriate target antigen specificity and signal sensitivity. The consistent and accurate tissue section staining patterns observed for each antibody between the widely used hapten-tagged method and the substantially faster H.O.H. Kit reinforces the point that neither assay specificity nor sensitivity would be sacrificed with the adoption of the Vector Laboratories' H.O.H. Kit. The H.O.H. Kit provides substantial time and cost savings in an alternative detection methodology that generates comparable assay performance by eliminating conjugation and antibody validation steps.

Comparison between H.O.H. Kit and antibody pre-complex method

To expand our study, we compared the H.O.H. Kit with the antibody pre-complex method outlined in reference 3. The pre-complex method, unlike the hapten-tagged approach, does not involve conjugating the primary antibody. This aspect of the pre-complex method makes it a potentially attractive alternative to the hapten-tagged method by saving time and associated costs of conjugation and validation.

From the data presented in Figures 4 and 5, however, the H.O.H. Kit provided superior signal-to-noise staining ratios in all tissues assayed compared with the antibody pre-complex method. From a critical point of view, we did not use the same reagents that the authors describe in reference 3, and we did not expend an inordinate amount of time trying to optimize that methodology. In following the paper's guidelines, though, we did obtain comparable results in our tissue sections to what the authors described in certain tissues they evaluated. The authors noted residual background staining in some tissue specimens that could not be eliminated with the pre-complex approach they developed. Additionally, as the authors stated, modifications to the described antibody pre-complex method would be required to further reduce or eliminate the persistent background staining. What those modifications

would be and how they would affect IHC assay time and costs were not speculated. As the antibody pre-complex method is presented in the paper, it would not be a viable alternative IHC approach compared with either the H.O.H. Kit or the hapten-tagged method for the assessment of human antibodies in TCR assays.

As indicated, the H.O.H. Kit uses an unconjugated primary antibody approach similar to the pre-complex method. The use of unmodified primary antibodies, particularly when evaluating potential therapeutic candidates, could be a preferred approach over conjugated, modified antibodies. In recognizing the opportunity to develop a more efficient method that mitigates the risks of using modified antibodies, Vector Laboratories developed highly refined reagents that incorporate aspects of the antibody pre-complex approach and combined them with a novel quenching solution. The outstanding tissue staining results and favorable abbreviated workflow presented here showcase that the H.O.H. Kit offers a superior alternative to current IHC methods used in assessing human primary antibodies on human tissue sections.

Summary

In this work, we present a novel immunohistochemical technology, designated the Human on Human (H.O.H.) Immunodetection Kit (catalog number: HOH-3000), for detecting human primary antibodies on frozen and FFPE human tissues. We obtained strong, specific signals and minimal background even in lymphoid tissues with high endogenous antibody content. Using two biosimilar therapeutic antibodies, anti-PD-1 (Pembrolizumab), anti-HER-2 (Trastuzumab), we saw excellent signal-to-noise ratios, with results comparable to detection via a hapten tag. The H.O.H. Kit technology works with multiple human isotypes and antibody configurations, including bivalent antibodies without Fc regions. This approach can accelerate safety testing and avoid potential issues associated with using modified antibodies in place of the actual drug in cross-reactivity assays.

Summary table comparing the three methods for detecting human primary antibodies on human tissue sections

	Little optimization required	Uses unmodified primary antibody	Saves significant time not having to validate tagged antibody	Good signal to noise staining ratio on frozen sections	Convenient, volume matched reagents
Vector H.O.H. Kit	X	X	X	X	X
Hapten-tagged antibody method				X	
Pre-complex method		X	X		

Published References

1. Leach, M.W. et al., Tox. Pathol., 38: 1138-1166, 2010
2. Points To Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, FDA, 1997 (web link: https://www.fda.gov/media/76798/download)
3. Goodpaster, T. et al., J. Histochem. Cytochem., Vol. 62(3):197-204, 2014
4. Takai, H. et al., ACTA Histochem., Vol. 113(4):472-476, 2011

Appendix 1: HOH-3000 User Guide

Kit Components

Product Name	Volume
Protein Block	5 mL
Solution A	2.5 mL
Solution B	2.5 mL
HRP Anti-Goat IgG	300 µL
HRP Antibody Diluent	5 mL
ImmPACT® DAB EqV Reagent 1 (Chromogen)	2.5 mL
ImmPACT® DAB EqV Reagent 2 (Diluent)	2.5 mL

Storage

Store reagents in original bottles at 2-8°C

PART 1: Human Antibody Solution Preparation

1. Determine the total volume of Human Antibody Solution required. Assume 100 µl per section.
2. Aliquot out a volume of Solution A equal to half the volume determined in Step 1.
3. Dilute humanized/human antibody in Solution A to twice the final concentration needed. Mix well.
4. Incubate 30-40 minutes at room temperature.
5. Add a volume of Solution B that equals the volume of Solution A used in Step 2. Mix well.
6. Incubate 30-35 minutes at room temperature.
7. The Human Antibody Solution is now ready for use.

NOTE: Timing the preparation of the Human Antibody Solution is important. It should be prepared such that it is ready for use after the Protein Block step (Step 4 of the Staining Procedure) is completed. If the Human Antibody Solution is not ready, the Protein Block time can be extended. Total Human Antibody Solution preparation time is about 1 hour.

Human Antibody Dilution Example:

Final human antibody dilution = 1/100

Total working volume needed = 1 mL (Preparation - Step 1)

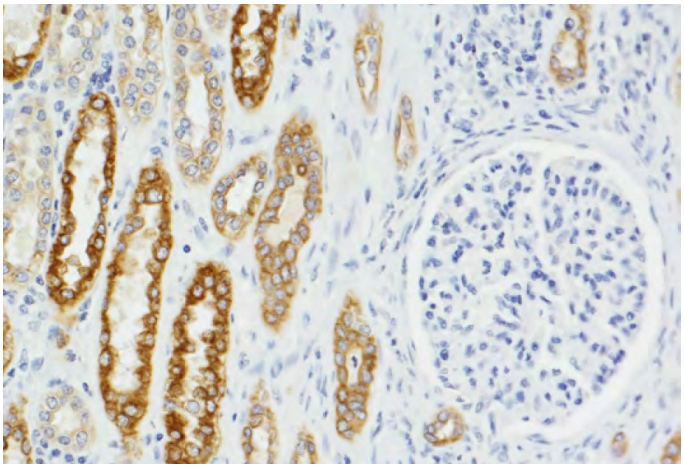
Solution A (Preparation - Step 2)	Human Primary Antibody (Preparation - Step 3)	Solution B (Preparation - Step 5)
0.5 mL	10 µL	0.5 mL

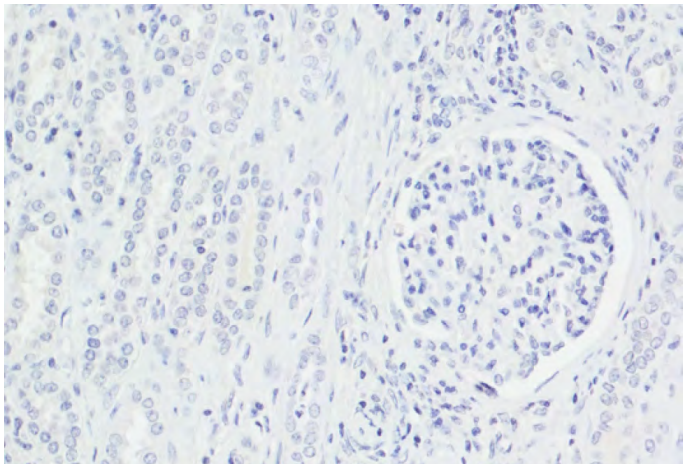
PART 2: Staining Procedure

Optimized for 4-6 µm thick sections

1. Prepare tissue sections as required by staining procedure.
2. Wash in tap water for 5 minutes.
3. Quench endogenous peroxidase activity if required. Wash in buffer for 5 minutes.
4. Incubate sections for 10-20 minutes in Protein Block. Tip off.
5. Apply the Human Antibody Solution (prepared in part 1) and incubate for 30-60 minutes.
6. Wash for 2 x 5 minutes in buffer.
7. HRP Anti-Goat IgG is provided at 0.5 mg/ml. Dilute to 20 µg/ml (1:25 dilution) in HRP Antibody Diluent. Apply the diluted HRP Anti-Goat IgG to sections and incubate for 15 minutes.
8. Wash for 2 x 5 minutes in buffer.
9. Combine equal volumes of ImmPACT DAB EqV Reagent 1 with ImmPACT DAB EqV Reagent 2. Mix well.
10. Incubate sections in the ImmPACT DAB EqV working solution until desired stain intensity develops, approximately 5-10 minutes.
11. Wash for 2 x 5 minutes in buffer.
12. Rinse sections in tap water.
13. Counterstain if desired, clear and mount.

NOTE: Signal-to- noise may be optimized by titering the human primary antibody in Solution A (Step 3 of Part 1), by varying the Primary Antibody incubation time (Step 5 of Part 2), or by varying the HRP Anti-Goat IgG concentration and incubation time (Step 7 of Part 2).





Serial sections of human kidney (FFPE). Left image shows strong, specific staining (brown) using human anti-cytokeratin primary antibody and detected with the H.O.H. Kit. Right image is a negative control showing absence of staining (no background) with the omission of just the primary antibody in the H.O.H. Kit assay. Both sections counterstained with hematoxylin (blue nuclei).

Appendix 2: Frequently Asked Questions

Q. Can I apply the H.O.H. Kit reagents to an open automated tissue staining platform?

A. After the primary antibody preparation steps, you can apply the secondary detection reagents using an automated platform. Since the incubation steps are brief, there may not be any time-savings advantage over a manual staining approach once the slides and reagents have been loaded and unloaded on the instrument.

Q. Is the H.O.H. Kit offered in a larger volume to accommodate a high throughput procedure while maintaining consistency with the same reagent lot?

A. Yes, as the manufacturer we can certainly provide all kit reagents in larger matching volumes. Please contact our Custom & OEM services for lead time and availability inquiries.

Q. Can the H.O.H. Kit be used to detect chimeric human antibodies?

A. Yes, this kit will detect chimeric human antibodies. The detection methodology was developed to ensure broad recognition of human IgG that is present on human, humanized, and chimeric primary antibodies. The CD20 antibody listed in Table 2 is a chimeric human primary antibody that was used as part of this study.

Q. Can the H.O.H. Kit be adapted for use in an immunofluorescence application?

A. The current kit reagent configuration utilizes peroxidase (HRP) enzyme in combination with a highly sensitive formulation of DAB. Modification of the kit and the optimized components for fluorescence visualization may compromise detection specificity or sensitivity, and as such, is not recommended or supported.

Q. If I observe some background staining in certain tissue sections, what parameters of the supplied IHC procedure shoul I optimize to eliminate the background?

A. Should background occur in some tissues, we suggest to initially alter the amount of primary antibody added during the preparation step, and/or, vary the time the primary antibody is incubated on the tissue sections. Additionally, the working concentration and incubation time of the HRP anti-goat conjugate may also be changed to improve the signal-to-noise staining ratio.

To learn more visit:
vectorlabs.com/HOH

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