WHITE PAPER

VectaPlex™ Antibody Removal Kit (VRK-1000)

Introduction

Multiplexing is a powerful technique that enables the extraction of detailed, spatially resolved information from tissue samples and provides deeper insights into intricate biological processes. Despite its widespread use, multiplexing faces challenges including cross reactivity between rounds of detection reagents and complex assay optimizations. To address this, antibody stripping processes were pioneered. Traditional methods for removing detection reagents such as Heat-Induced Epitope Retrieval (HIER) or use of reductants, surfactants, or pH alterations, often compromise sample integrity and fail to fully remove detection reagents which can lead to non-specific staining. VectaPlex overcomes these challenges by providing an efficient and reliable solution for removing antibodies and other detection reagents from tissue samples. This innovative approach eliminates the limitations of traditional methods allowing researchers to perform multiple staining rounds without lengthy optimization procedures while preserving antigenicity and tissue morphology. Vectaplex can be used in Immunohistochemistry (IHC), Immunofluorescence (IF), or Tyramide Signal Amplification (TSA) workflows.

Background

Multiple methods have been used to remove bound detection reagents, but there is not a standard protocol that has been widely adopted. HIER methods have become the industry standard even though they have many drawbacks such as: i) damaging the tissue, ii) altering the antigenicity of epitopes and iii) failing to completely remove antibodies and other detection reagents. VectaPlex avoids these drawbacks as it is an easy-to-use, gentle, and effective treatment that removes antibodies and other non-covalently bound detection reagents. See **Figure 1** for some examples of the impacts to tissue morphology from various HIER methods compared to VectaPlex.

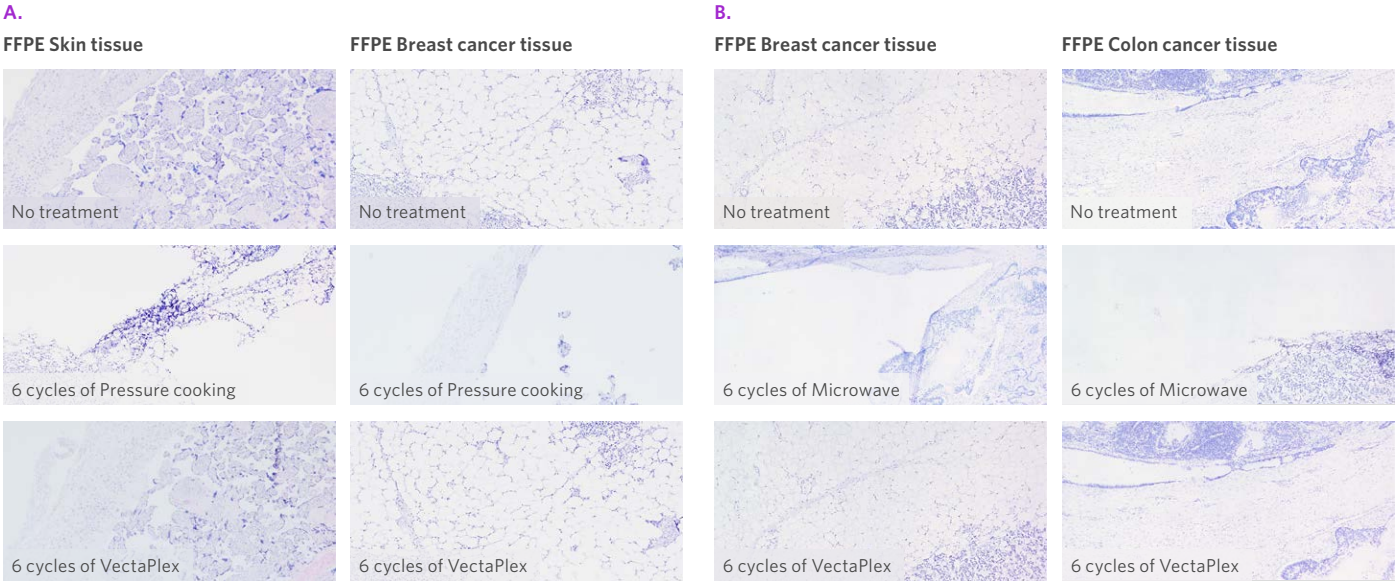
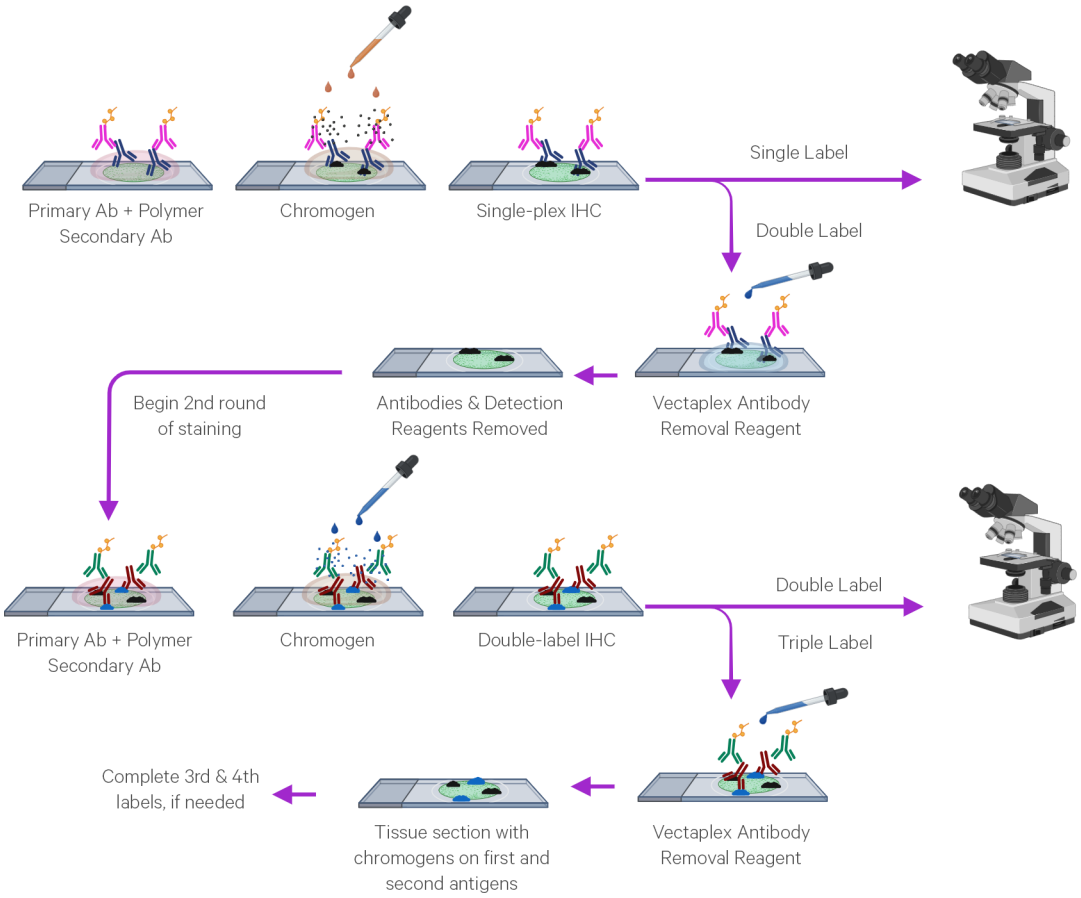


***Figure 1**: VectaPlex preserves tissue antigenicity and morphology more than (A) pressure cooking or (B) microwave treatment. Images of tissue damage as shown by hematoxylin staining before any treatment (top row), after 6 rounds of microwave/pressure cooking (middle row) and by VectaPlex (bottom row). VectaPlex preserves tissue morphology while other methods show extensive tissue damage.*

Another approach for achieving multiplexing in IHC/IF is the direct labeling of multiple primary antibodies. However, this method has notable drawbacks, including the potential to affect the antibody's affinity or specificity, the high cost and labor-intensive nature of the labeling process, and the potential for significantly low yields when achieving the desired purity. VectaPlex eliminates these limitations by allowing researchers to use any unlabeled antibody as their primary binder, while supporting any detection modality, including IHC, IF, and TSA workflows.

Some IHC/IF multiplexing platform technologies rely on specialized equipment or protein tags for labeling multiple targets. However, these methods often demand extensive resources for data analysis and labelling of detection reagents which are cost-prohibitive for many users. Additionally, these techniques typically require significant optimization, as the removal process for the detection reagents can impact markers differently based on their order or position in the assay, making the workflow both cumbersome and expensive. VectaPlex addresses these challenges by allowing users to work with their markers in any order and with any color. It preserves the integrity of fluorescent and tyramide dyes and is fully compatible with the chromogenic substrates listed in Table 1 such as ImmPACT® DAB, ImmPACT® Vector® Red, and Vector® Blue.

IHC Workflow



VectaPlex has now been validated for use in IHC workflows, and Vector Labs has qualified many of its chromogenic substrates with the VectaPlex treatment. Brown HRP substrates, including Vector DAB (SK-4100), ImmPACT DAB (SK-4105), and ImmPACT DAB EqV (SK-4103), red AP substrates including Vector Red (SK-5100), ImmPACT Vector Red (SK-5105), and Vector Red EqV (SK-5103), and blue AP substrates including Vector Blue (SK-5300), are compatible with VectaPlex treatment (see Table 1). This provides users the flexibility needed to achieve IHC 4-plex staining easily when combined with a fourth substrate color of their choice, since the last substrate will not interact with VectaPlex. We recommend ImmPACT SG (SK-4705) for grey or ImmPACT VIP (SK-4605) for purple as the fourth substrate color as they provide good contrast to the brown, blue, and red substrates already deposited. Additionally, other substrates can be evaluated for compatibility with VectaPlex (see FAQ section). Compatible substrates can be used in any order and do not require re-optimization if staining order is changed.

Substrate	SKU	Color	Detection Enzyme
Vector DAB	SK-4100	Brown	HRP
ImmPACT DAB	SK-4105	Brown	HRP
ImmPACT DAB EqV	SK-4103	Brown	HRP
Vector Red	SK-5100	Red	AP
ImmPACT Vector Red	SK-5105	Red	AP
ImmPACT Vector Red EqV	SK-5103	Red	AP
Vector Blue	SK-5300	Blue	AP

***Table 1**: A list of the approved chromogenic substrates that are compatible with VectaPlex products.*

In **Figures 2 and 3**, VectaPlex was used to strip 3 times and create a 4-plex image. CD68 was stained first using ImmPACT DAB (SK-4105) to give the brown color, CD20 was stained second using ImmPACT Vector Red (SK-5105) to give the red color, CD34 was stained third using Vector Blue (SK-5300) to give the blue color, and Desmin was stained fourth using ImmPACT SG (SK-4705) to give the grey color.

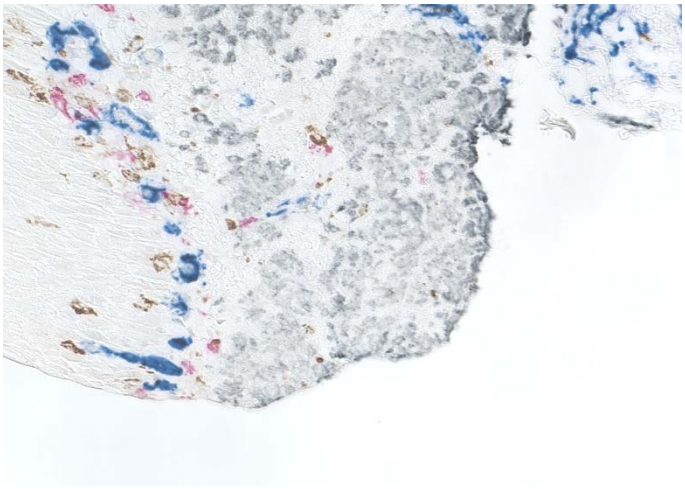


***Figure 2**: 20x magnification of Esophagus tissue stained for CD68 (brown), CD20 (red), CD34 (blue), and Desmin (gray).*

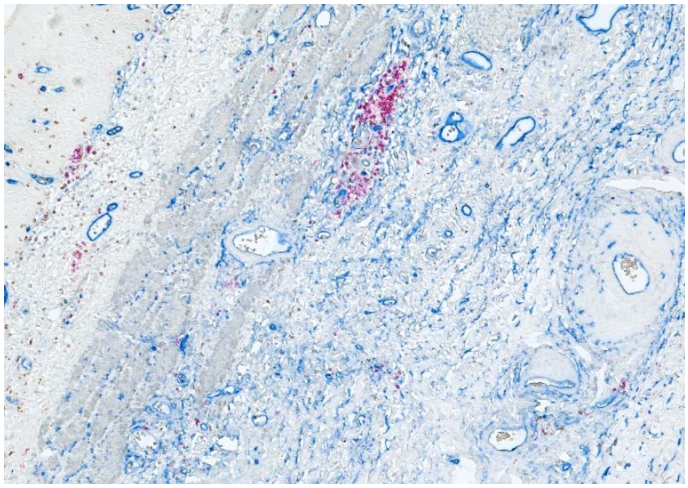
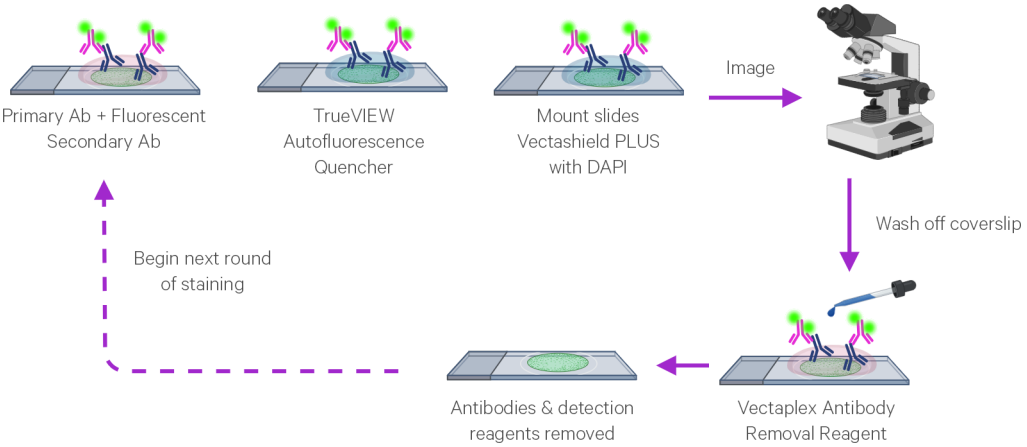


***Figure 3**: 4x magnification of Esophagus tissue stained for CD68 (brown), CD20 (red), CD34 (blue), and Desmin (gray).*

IF Workflow



Multiplex immunofluorescence has emerged as a powerful and versatile technique in the field of spatial biology and biomedical research enabling the visualization of multiple biomolecules within a single sample. This method provides invaluable insights into complex cellular processes and disease mechanisms facilitating a deeper understanding of intricate molecular interactions. However, researchers performing multiplex IF face challenges including cross-reactivity between detection reagents and limited fluorescent channels on equipment for image capture. VectaPlex eliminates these concerns from the workflow. By removing the antibodies and other non-covalent reagents between staining rounds, cross-reactivity between reagents is eliminated. Additionally, the workflow allows the use of a single fluorophore for each round of detection since resulting images can be false-colored and aligned using software such as Image J[1].

Epitope	False Color
CD20	Red
CD34	Yellow
Desmin	Cyan
AE1/AE3	Magenta
CD3	Gray
Vimentin	Green
DAPI	Blue

***Table 2**: List of the epitopes that were stained and false colors used in Figures 4 and 5.*

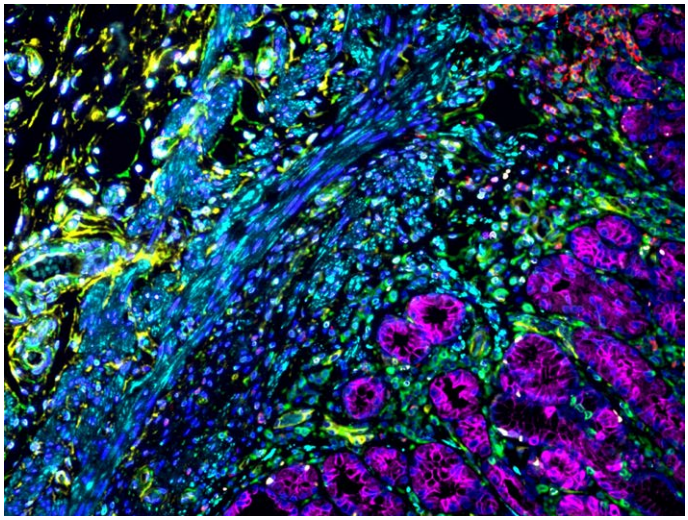
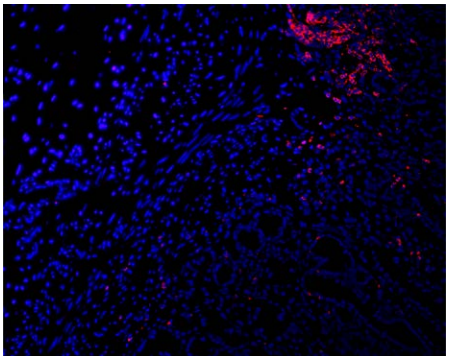
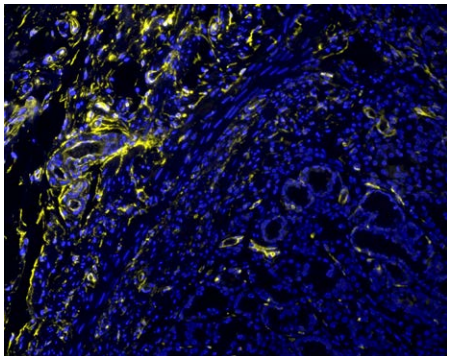


***Figure 4**: Multiplex image showing positive staining of 6 mouse primary antibodies and DAPI on FFPE stomach tissue. Standard immunofluorescence workflows were used with VectaPlex and all detection was performed horse anti-mouse 488. Epitopes stained and false colors used are shown in **Table 2**.*

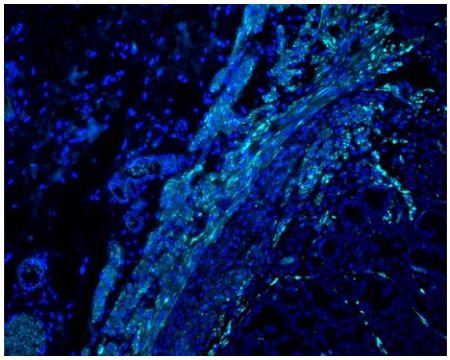
***Figure 5**: Individual images merged to create the multiplex image shown in Figure 4. Primary antibodies that were used are listed in A-F and Table 2. All tissues are stained with DAPI.*



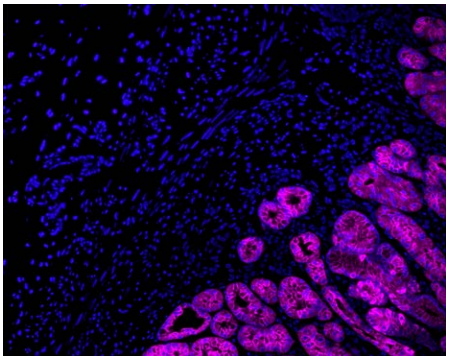
A. CD20 staining shown in red.



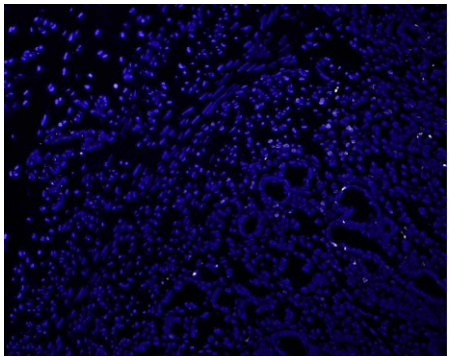
B. CD34 staining shown in yellow.



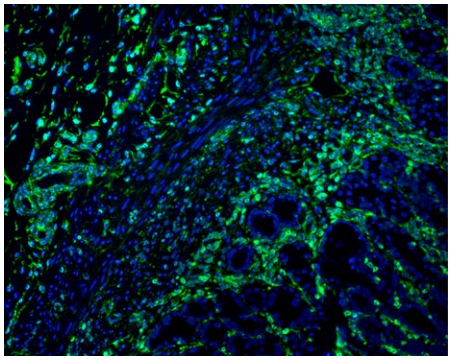
C. Desmin staining shown in cyan.



D. AE1/AE3 staining shown in magenta.



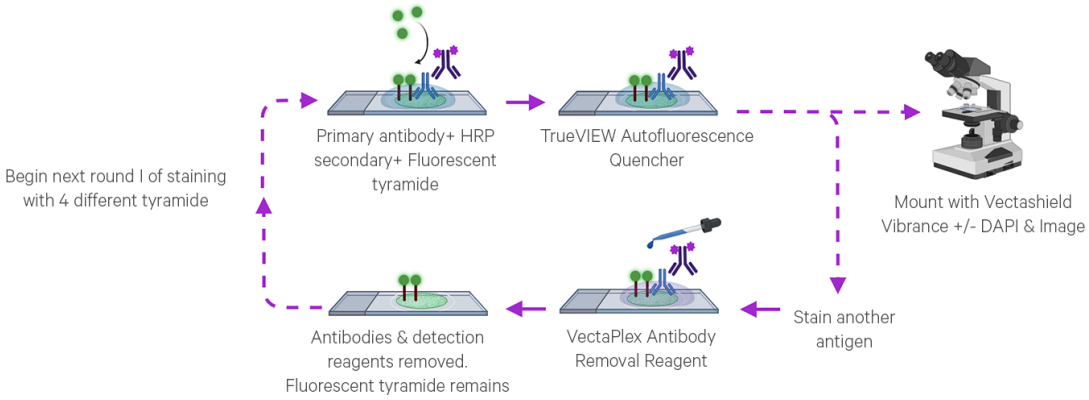
E. CD3 staining shown in gray.



F. Vimentin staining shown in green.

TSA Workflow

Tyramide Signal Amplification (TSA) is a powerful tool that allows multiple markers to be detected in a single tissue by using a fluorescent readout. By covalently attaching the signal molecule or fluorophore to the surface, sequential rounds of staining can be completed once the remaining detection reagents are removed. However, impacts to tissue morphology and target antigenicity combined with incomplete removal of detection reagents often limit the effectiveness of this workflow. When TSA is paired with VectaPlex, these issues are eliminated.



Conclusion

The benefits of using VectaPlex in IF have now been expanded into the IHC realm when using the approved substrates. With these new methods available, users can now strip their detection reagents from the tissue with VectaPlex and achieve chromogenic IHC staining in a multiplex manner. The benefits of preserving tissue integrity, efficient stripping of detection reagents, and ease-of-use are now available in IHC when using VectaPlex. By stripping away detection reagents, researchers are now able to extract more information from each sample. Through observation of the spatial organization of multiple markers at the same time, new insights can be drawn. Now, explore many epitopes in a single experiment using VectaPlex and increase your through-put, conclusions and correlations. With a single kit, take your single-plex and turn it into multiplex.

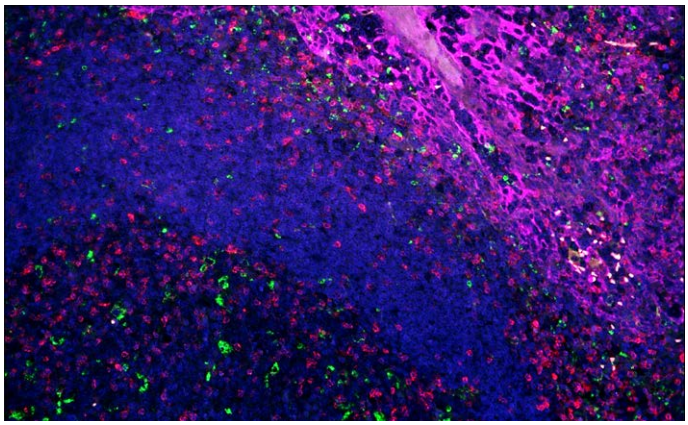


***Figure 6**: Tyramide Signal Amplification based on multiplexing using VectaPlex and 3 mouse primary antibodies. CD3 staining in red (Opal 570), CD68 in green (Opal 520), AE1/AE3 in purple (Opal 690) and DAPI staining in blue. Performed on FFPE tonsil tissue.*

Protocols for Use:

Immunohistochemistry (IHC) Workflow

1. Complete standard chromogenic staining procedure using any of the approved substrates (see Table 1) for the first marker.
2. Add sufficient VectaPlex Reagent A to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
3. Wash slides briefly with PBS.
4. Add sufficient VectaPlex Reagent B to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
5. Wash slides with PBS for 5 minutes.
6. The tissue section is now ready to begin another IHC staining procedure with a protein blocking step followed by the next round of antibody staining. Each round of stripping and staining should use one of the different approved substrates that are compatible with VectaPlex (see Table 1).
7. Repeat until the desired number of antigens have been detected. The final chromogenic stain can be with any substrate since it will not contact VectaPlex. ImmPACT SG (SK-4705, grey) or ImmPACT VIP (SK-4605, purple) will work well with the brown, red and blue of the previous stains.

Immunofluorescence (IF) Workflow

1. Complete standard immunofluorescence staining procedure. Mount with a non-hardening (non-setting) mounting medium and image slide such as VECTASHIELD® Plus Antifade Mounting Media with DAPI (H-2000).
2. Remove coverslip from tissue section and wash thoroughly with PBS pH 7.4 to remove residual mounting medium.
3. Add sufficient VectaPlex Reagent A to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
4. Wash slides briefly with PBS.
5. Add sufficient VectaPlex Reagent B to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
6. Wash slides with PBS for 5 minutes.
7. The tissue section is now ready to begin another immunofluorescence staining procedure with a protein blocking step followed by the next round of antibody staining, mounting, and imaging.
8. Repeat until the desired number of antigens have been detected.
9. Align your individual images and false-color them as needed.

Tyramide Signal Amplificatio (TSA) Workflow

1. Complete standard tyramide signal amplification staining procedure.
2. Add sufficient VectaPlex Reagent A to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
3. Wash slides briefly with PBS.
4. Add sufficient VectaPlex Reagent B to cover tissue section completely (typically ~150–200 ×L) and incubate for 15 minutes at room temperature.
5. Wash slides with PBS for 5 minutes.
6. Tissue sections are now ready to begin another fluorescent tyramide signal amplification staining procedure with a protein blocking step followed by the next round of antibody staining using a differently colored dye.
7. Repeat until the desired number of antigens have been detected.
8. Once staining is completed, mount tissue sections and image.

Frequently Asked Questions

FAQ | Compatibility with Other Products and Reagents

Q1 **Can other substrates be used with VectaPlex?**

A Perhaps. We have not tested substrates from other vendors, but they may be compatible. To test this, simply stain two sections with the substrate in question and treat one of them with VectaPlex. If the staining intensity is unchanged, the substrate should be compatible with VectaPlex.

Q2 **I am using TrueView to quench autofluorescence; will TrueVIEW be stripped by VectaPlex reagents?**

A We used TrueView in our VectaPlex IF workflow to suppress autofluorescence. Application of VectaPlex removes some, but not all, of the TrueView reagent. Since some TrueView remained after VectaPlex application, we did not add TrueView in every cycle, but only as needed to suppress autofluorescence.

Q3 **Does VectaPlex work for all species of antibodies?**

A VectaPlex has been tested with mouse and rabbit primaries, and with horse and goat secondaries. We expect it to work well with other species of antibodies as well.

Q4 **Is VectaPlex compatible with all antibodies?**

A VectaPlex has been tested on more than 30 common antibodies and has worked on them without issue.

Q5 **Does VectaPlex work on different tissue types?**

A VectaPlex has been tested on and works well with various FFPE tissues, including FFPE breast and lung tissue which are very fragile.

FAQ | Application and Usage Guidelines

Q1 **Can I use VectaPlex on frozen sections?**

A No. During development, VectaPlex was optimized for FFPE tissues, where the power to remove antibodies at room temperature was balanced with the need to preserve tissue morphology. On frozen tissues, VectaPlex treatment can lead to tissue damage and loss.

Q2 **Is there a maximum number of times you can use VectaPlex on one section?**

A VectaPlex has been tested for up to 6 cycles with no adverse effects. We don't expect there to be issues with increasing the number of cycles, but we suggest testing with controls for adequate comparisons. We recommend running experiments to confirm that the use of more cycles does not impact the antigenicity or tissue morphology.

Q3 **Can VectaPlex be used on an autostainer?**

A Yes. We have validated this product for use on automated systems.

Q4 **In an IF protocol, will the VectaPlex Antibody Removal Kit also strip the autofluorescence from the tissue?**

A VectaPlex does not appear to reduce autofluorescence background.

Q5 **Is there a protocol for removal of coverslips between VectaPlex cycles when performing IF?**

A We recommend using VECTASHIELD® Plus Antifade Mounting Media with DAPI (H-2000), which is an aqueous non-hardening mounting medium. After each imaging cycle, the coverslip can be easily removed by incubating in PBS and the residual VECTASHIELD® is removed with a PBS wash.

FAQ | Storage and Stability

Q1 **I accidentally put my VectaPlex kit in the freezer. Can I still use it?**

A Yes. VectaPlex can withstand a freeze-thaw cycle and still performs as expected. However, the recommended storage temperature is at ambient (15-25 °C). Additionally, the kit will still perform properly if placed at 4 °C.

Important Notes:

1. Mixing VectaPlex reagents with bleach may result in the formation of a hazardous gas. For this reason, do not mix reagents or waste solutions from this process with bleach, and use reagents in a well-ventilated area using appropriate laboratory personal protective equipment (PPE).

2. When performing the immunofluorescence workflow, images from successive staining cycles can be registered and overlaid using free software such as Fiji2. A protocol for image alignment is also available on the product page. https://vectorlabs.com/productattachments/protocol/Guidance-for-alignment-of-images-to-generate-multiplex-images-Immunofluorescence.pdf

3. When performing the immunofluorescence workflow, use of a mounting medium containing DAPI during each staining cycle facilitates image alignment.

4. This product has been developed for use across a wide range of FFPE sample and staining conditions. Up to six rounds of successive staining and antibody removal have been demonstrated. However, performance may vary depending on the sample or experimental design being used. For this reason, control experiments using single-plex staining with and without VectaPlex usage are recommended.

For Research Use Only. Not intended for animal or human therapeutic use.

References

1. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012 Jul;**9(7)**:671-5. [PubMed]
2. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012 Jun 28;**9(7)**:676-82. [PubMed]

Get in touch with us at
customerservice@vectorlabs.com
to take the next step in your bioconjugate journey.

vector LABORATORIES
vectorlabs.com