WHITE PAPER

Use of Vector Laboratories' VectaPlex™ Antibody Removal Kit on Leica BOND RX Automated Stainer

Abstract

Multiplex tissue staining is an essential tool in spatial biology, translational research, and biomarker discovery, enabling the simultaneous visualization of multiple biomarkers within a single tissue section. However, conventional multiplexing workflows are often limited by antibody cross-reactivity, epitope degradation, and rigid staining order requirements, challenges that are amplified in automated staining environments.

VectaPlex™ Antibody Removal Technology enables efficient removal of non-covalently bound antibodies and detection reagents while preserving tissue morphology and antigen integrity. This capability allows sequential staining without signal carryover or tissue damage.

Here, we demonstrate the use of the VectaPlex™ Antibody Removal Kit on the Leica BOND RX automated staining platform for high-plex immunohistochemistry (IHC). Integration of VectaPlex enables reproducible, walk-away multiplex staining with reduced optimization time, flexible marker ordering, and improved signal fidelity. This work demonstrates how VectaPlex transforms multiplex staining from a manual, optimization-intensive process into a scalable and automated workflow suitable for translational research and discovery applications.

Introduction

Multiplexed tissue imaging has become a cornerstone of modern spatial biology, enabling simultaneous visualization of multiple biomarkers within a single tissue section. This capability is critical for understanding cellular heterogeneity, biomarker co-localization, and tissue architecture in both basic research and translational studies.

Conventional multiplexing approaches face limitations such as antibody cross-reactivity and harsh stripping conditions, which can compromise tissue morphology and antigen integrity and produce confounding results due to signal carryover between sequential staining rounds. Common antibody removal methods, including heat-induced epitope retrieval, extreme pH exposure, or reducing agents, may result in incomplete antibody removal, cumulative tissue damage, or progressive loss of antigenicity with repeated use. These limitations often necessitate strict control of staining order and extensive assay optimization, increasing development time and reducing reproducibility.

VectaPlex™ Antibody Removal Technology was developed to overcome these challenges. VectaPlex™ selectively removes non-covalently bound antibodies and detection reagents while preserving tissue architecture and deposited chromogenic signal. This enables true sequential staining without harsh stripping conditions or rigid marker ordering constraints.

In this application note, we describe the evaluation of VectaPlex on the Leica BOND RX automated staining platform, demonstrating a robust and scalable solution for multiplex IHC workflows. The robust performance observed in this automated setting further suggests that VectaPlex may be readily extended to other automated multiplexing modalities, including immunofluorescence and fluorescent tyramide amplification workflows. Supporting this broader applicability, a previously published application note demonstrated that VectaPlex preserves antigenicity and tissue morphology across six sequential staining cycles. Readers are referred to this prior application note for detailed evaluation of antigen preservation, tissue integrity, and implementation of VectaPlex in manual multiplex workflows, including chromogenic IHC, immunofluorescence, and fluorescent tyramide signal amplification.

Key Advantages of VectaPlex Technology

- Efficient removal of non-covalently bound antibodies
- Preservation of tissue morphology and antigen integrity
- Compatible with chromogenic IHC and immunofluorescence
- Eliminates antibody carryover between staining cycles
- Enables flexible marker ordering
- Reduces assay development and optimization time
- Compatible with automated staining workflows

Leica BOND RX

The Leica BOND RX is a widely adopted automated staining platform designed for high-throughput applications including IHC and IF. As an open system, the BOND RX supports the use of externally supplied reagents in addition to vendor-provided solutions, allowing laboratories to implement custom workflows and advanced chemistries.

Its enclosed fluidics, programmable reagent handling, and precise temperature control make it well suited for multiplex staining when paired with a compatible antibody removal strategy. VectaPlex integrates seamlessly into the BOND RX workflow, enabling automated stripping at room temperature without repeated antigen retrieval or aggressive chemical treatment.

This integration allows users to perform multi-round staining with minimal tissue degradation, high reproducibility, reduced optimization burden, and full flexibility in reagent and target selection.

Materials and Methods

Reagent Configuration and Instrument Setup

All reagents used in this study, including the VectaPlex™ Antibody Removal Kit and associated detection reagents, were onboarded and optimized in accordance with Leica's recommended procedures for the BOND RX automated staining platform. Reagents were configured within the BOND software as Open or Titration reagents, allowing precise control over dispense volume, incubation time, and wash conditions to ensure optimal performance and reproducibility.

Optimization was performed following standard Leica guidelines to ensure:.

- Uniform tissue coverage with dispensed reagents
- Reagent performance equivalent to manual workflows
- Reproducibility across staining runs

Primary antibodies were optimized in single-plex assays to establish appropriate working concentrations for the multiplex workflow. No re-optimization based on staining order was required, as VectaPlex does not alter antigen integrity and therefore avoids the order-dependent effects commonly introduced by conventional antibody removal methods.

Dispense volumes of 150–200 µL were used to ensure complete coverage of tissue sections beneath the covertile.

For this study, FFPE tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval prior to loading onto the Leica BOND RX as this was found to provide the most sensitive detection of target antigens.

Table 1. Detection Reagents Used

Reagents	SKU
Vector Antigen Unmasking Solution (pH 6)	H-3300-250
BLOXALL Endogenous Blocking Solution, (HRP and AP)	SP-6000-100
2.5% Normal Horse Serum	S-2012-50
ImmPRESS HRP Universal Antibody (Horse Anti-Mouse/Rabbit IgG) Polymer Detection Kit	MP-7500
ImmPRESS-AP Horse Anti-Mouse IgG Polymer Detection Kit	MP-5402
Vector Blue Substrate Kit, Alkaline Phosphatase (AP)	SK-5300
ImmPACT Vector Red Substrate, Alkaline Phosphatase (AP)	SK-5105
ImmPACT SG Substrate, Peroxidase (HRP)	SK-4705
VectaPlex™ Antibody Removal Reagent	VRK-1000

Reagents	SKU
Methyl Green Counterstain	H-3402-500
VectaMount® PT Permanent Mounting Medium	H-5600-60

Once optimized, all parameters were incorporated into the finalized assay protocol (Table 2), and consistent performance was verified across multiple staining runs, tissue specimens, and target antigen combinations.

Table 2: Optimized Assay Protocol

Step	Reagent	Time	Temp
1	BLOXALL® Endogenous HRP/AP Blocking Solution	10 min	Room Temp
2	Protein Block (2.5% Normal Serum)	20 min	Room Temp
3	Primary Antibody	30 – 60 min	Room Temp
4	ImmPRESS HRP or AP Polymer Secondary Ab	15 - 30 min	Room Temp
5	BOND Wash/ PBS Wash	5 x 2 min	Room Temp
6	Chromogenic Substrate	5 – 10 min	Room Temp
7	VectaPlex Reagent A	15 min	Room Temp
8	BOND Wash/ PBS Wash	2 min	Room Temp
9	VectaPlex Reagent B	15 min	Room Temp
10	BOND Wash/ PBS Wash	5 min	Room Temp

The optimized protocol in Table 2 provides guidelines for the chromogenic staining of a single target antigen followed by the VectaPlex Antibody Removal treatment. Steps 2-10 can be repeated for additional staining cycles. For the final staining round, steps 7-10 can be omitted, and slides can be counterstained with a compatible counterstain (if desired), dehydrated and mounted.

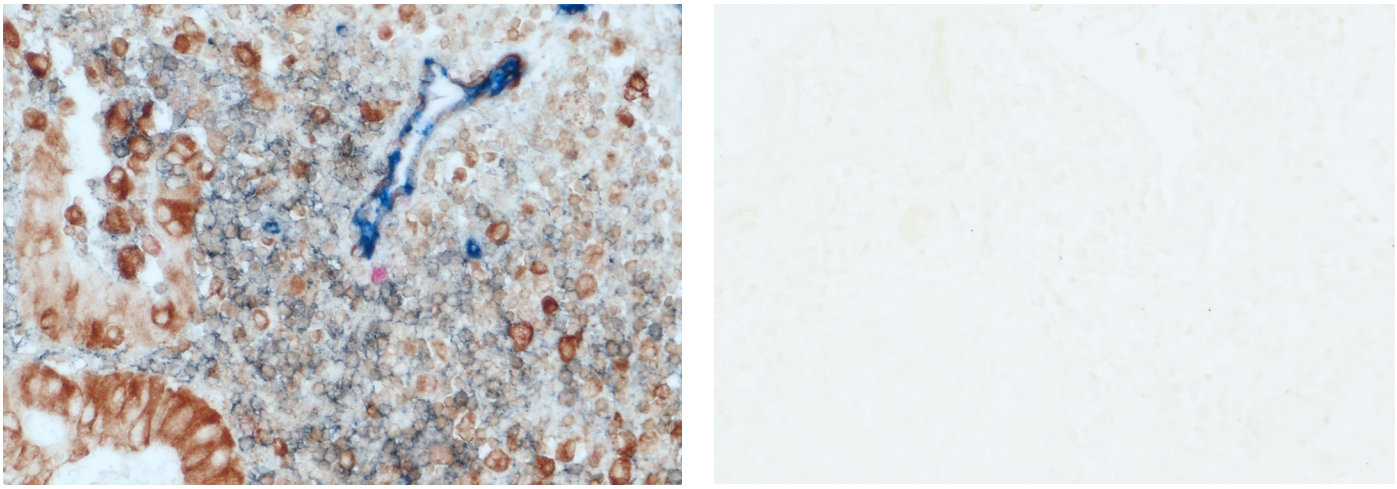
Multiplex staining performance was evaluated across multiple human tissue types. Four sequential staining cycles were performed using the following antibody panel:

Primary	Detection	Substrate
Mouse anti CD34	ImmPRESS AP anti-Mouse IgG	Vector Blue
Rabbit anti-Vimentin	ImmPRESS HRP anti-Rabbit IgG	ImmPACT DAB
Rabbit anti-S100	ImmPRESS AP anti-Rabbit IgG	ImmPACT Vector Red
Mouse anti-CD20	ImmPRESS HRP anti-Mouse IgG	ImmPACT SG

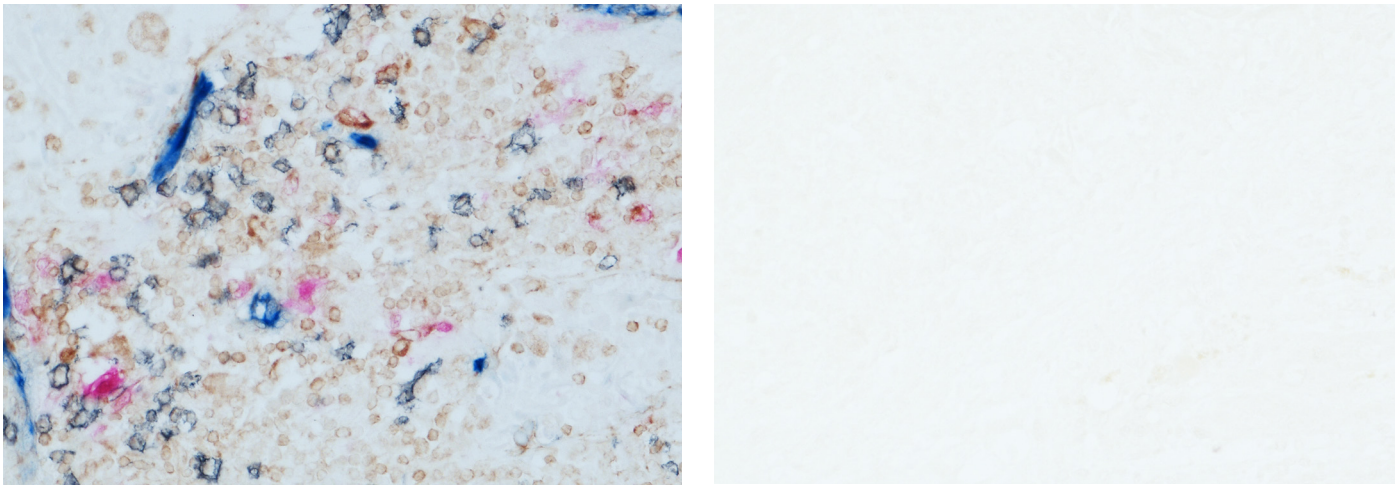
Results

Across all tissue types evaluated, staining performance remained consistent over sequential staining cycles. Tissue architecture was preserved, with no observable structural disruption or evidence of cumulative damage following repeated VectaPlex treatment. Antigen-specific staining intensity and spatial localization remained well defined across successive rounds, indicating preservation of antigen integrity and sustained detection efficiency over multiple cycles. No progressive increase in background signal or evidence of signal bleed-through was observed, consistent with effective removal of antibody and detection components without introduction of nonspecific staining or residual detection artifacts.

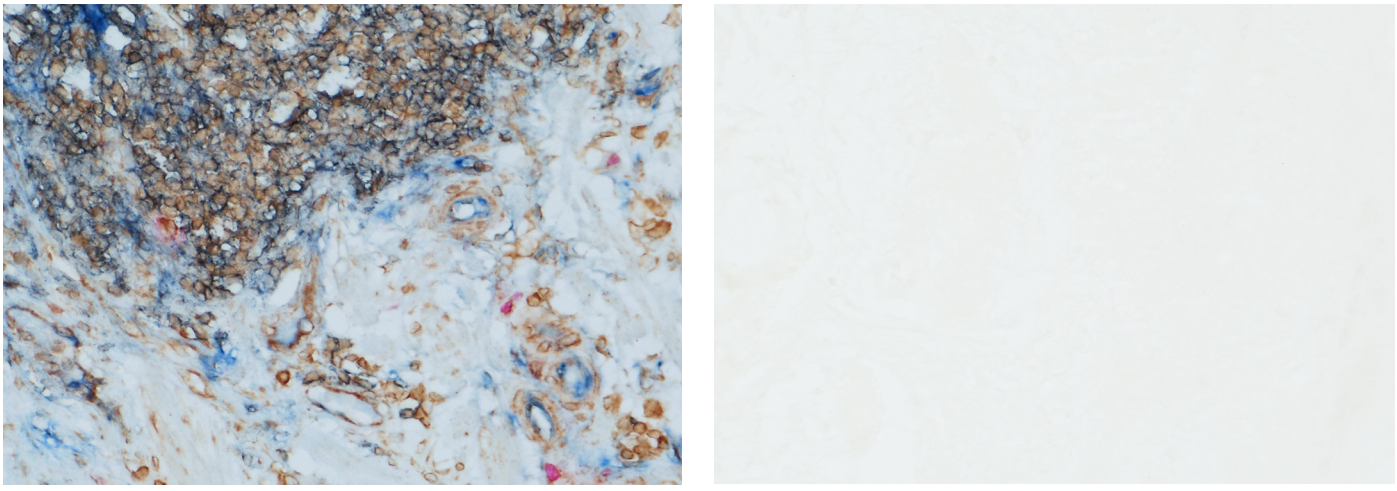
Collectively, these findings demonstrate that the key performance attributes previously observed in manual VectaPlex workflows, preservation of tissue morphology, maintenance of antigen integrity, efficient antibody removal, and absence of cumulative background, are reproducibly maintained on the automated Leica BOND platform. This confirms that VectaPlex supports reliable multi-round staining in automated multiplex immunohistochemistry workflows and is well suited for high-plex applications requiring consistent staining quality across sequential detection cycles.



***FFPE Human Uterine Carcinoma**: Left picture: Four rounds of staining using Ms anti-CD34 (blue), Rb anti-Vimentin (brown), Rb anti-S100 (red), Ms anti-CD20 (gray) . Right picture: Four rounds of staining without primary antibodies. Whole frame with 20× objective.*



***FFPE Human Squamous Carcinoma**: Left picture: Four rounds of staining using Ms anti-CD34 (blue), Rb anti-Vimentin (brown), Rb anti-S100 (red), Ms anti-CD20 (gray) . Right picture: Four rounds of staining without primary antibodies. Whole frame with 20× objective.*



***FFPE Human Stomach**: Left picture: Four rounds of staining using Ms anti-CD34 (blue), Rb anti-Vimentin (brown), Rb anti-S100 (red), Ms anti-CD20 (gray) . Right picture: Four rounds of staining without primary antibodies. Whole frame with 20× objective.*

Best Practices for Performing Multiplex IHC on the Leica BOND RX Using VectaPlex

As with any complex workflow, careful attention to assay design and execution is critical. The following considerations highlight key factors for successful implementation of the VectaPlex Antibody Removal Kit on the Leica BOND RX:

- **Workflow Setup:**
  Deparaffinization and antigen retrieval may be performed either manually prior to loading slides onto the Leica BOND RX or using the onboard BOND Dewax and Antigen Retrieval solutions. Both approaches are fully compatible with the VectaPlex workflow, and users are encouraged to evaluate each method to determine which provides optimal performance for their specific tissue types and target antigens.

- **Detection System Selection:**
  Detection systems should be selected based on the species and isotype of the target antibodies. Not all chromogenic substrates are compatible with the VectaPlex antibody removal chemistry. We have provided a list of validated substrates (see Table 3). Users wishing to employ alternative substrates should validate that their chromogenic signal is not impacted by exposure to the VectaPlex reagents.

Table 3: Chromogenic Substrates Compatible with VectaPlex Treatment:

Substrate	SKU	Detection Enzyme	Color	BOND Rx Incubation Time	Working Solution Stability (RT)
Vector DAB	SK-4100	HRP	Brown	5-8 min	6 hours
ImmPACT DAB	SK-4105	HRP	Brown	5-8 min	5 days
ImmPACT DAB EqV	SK-4103	HRP	Brown	5-8 min	3 days
Vector Red	SK-5100	AP	Red	15-20 min	1 hour
ImmPACT Vector Red	SK-5105	AP	Red	15-20 min	1 hour
Vector Blue	SK-5300	AP	Blue	15-20 min	4 hours

- **Final Substrate Considerations:**
  The final chromogenic substrate applied in the workflow is not exposed to the VectaPlex treatment and may therefore be any substrate appropriate for the detection enzyme, for example, ImmPACT SG Substrate Kit (SK-4705) was used in this study. Optimize incubation time for alternative substrates to ensure optimal performance.

- **Reagent Preparation:**
  Most reagents used for this automated, multiplex workflow are ready-to-use solutions that do not require mixing or diluting. However, the chromogenic substrates must be mixed onboard or loaded as pre-mixed working solutions. When using pre-mixed substrates, users should verify that the working solution is stable for the full duration of the staining protocol to ensure maximum signal development and consistent, optimal staining performance. See table 3 above for working solution stabilities for substrates compatible with VectaPlex treatment. Vector Red and ImmPACT Vector Red should be mixed on board due to the short working solution stability.

- **Assay Optimization:**
  Individual antibodies should be optimized as single-plex assays prior to multiplex implementation to confirm signal intensity, specificity, and background performance.

- **Target Proximity Considerations:**
  When target antigens are expressed in close proximity (e.g., within the same cellular compartment), the order of detection should be evaluated, as chromogenic deposition may introduce steric effects that influence subsequent antibody access.

- **Controls:**
  Standard IHC controls, including positive controls, negative controls, chase control, and deletion controls, should be evaluated during assay development to confirm specificity, reproducibility, and overall assay performance.

Conclusion

VectaPlex™ Antibody Removal Technology enables robust, reproducible multiplex staining on the Leica BOND RX by overcoming the limitations of traditional stripping methods. By preserving tissue morphology, maintaining antigen integrity, and ensuring complete antibody removal between staining cycles, VectaPlex eliminates the need for rigid staining order optimization and significantly reduces assay development time.

Integration with the Leica BOND RX transforms multiplex staining from a technically demanding, manual process into a streamlined and reproducible automated workflow. This enables reliable high-plex IHC and IF applications for translational research, biomarker discovery, and spatial biology studies.

The robust performance observed in this automated setting further suggests that VectaPlex may be readily extended to other automated multiplexing modalities, including immunofluorescence and fluorescent tyramide amplification workflows.

References

1. **White Paper:** Use of Vector Laboratories ImmPRESS® Polymer IHC Detection Reagents on Open Automated Staining Platforms
2. **White Paper:** VectaPlex™ Antibody Removal Kit (VRK-1000)
3. **User Guide:** VectaPlex™ Antibody Removal Kit
4. **User Guide:** Guidance for Image Alignment

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