USER GUIDE

VectaPlex™ Antibody Removal Kit

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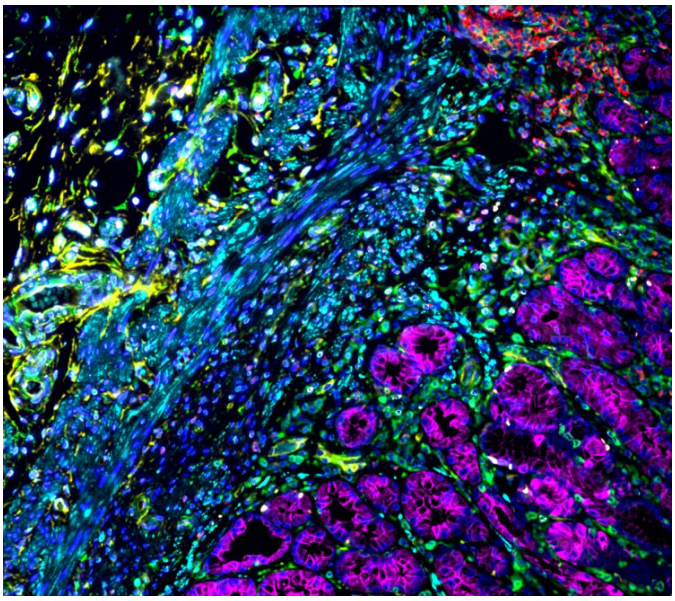
Cat. No. VRK-1000

Storage Ambient (15–25° C)

Description This ready-to-use kit is designed to remove antibodies and other non-covalently bound detection reagents from formalin-fixed paraffin-embedded (FFPE) tissue sections in immunohistochemistry, immunofluorescence or tyramide signal amplification staining workflows.

Components

Product Name	Solution
VectaPlex™ Antibody Removal Reagent A	25 ml
VectaPlex™ Antibody Removal Reagent B	25 ml



Stomach section stained with 6 mouse primary antibodies (CD20 (Red), CD34 (Yellow), DES (Cyan), AE1/AE3 (Magenta), CD3 (Grey), VIM (Green)) detected with DyLight™ 488 horse anti-mouse IgG and mounted with VECTASHIELD® PLUS with DAPI. This composite image was generated from 6 rounds of immunofluorescent staining using the VectaPlex™ Antibody Removal Kit.

Protocols for Use:

Immunohistochemistry (IHC) Workflow

1. Complete standard chromogenic staining procedure using validated substrate (see Table 1) for the first marker.
2. Add sufficient VectaPlex™ Reagent A to cover tissue section completely (typically ~ 150–200 uL) 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 uL) 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 a validated substrate that is 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 such as VECTASHIELD® Plus Antifade Mounting Media with DAPI (H-2000) and image slide.
2. Remove coverslip from tissue section and wash thoroughly with PBS to ensure complete removal of residual mounting medium.
3. Add sufficient VectaPlex™ Reagent A to cover tissue section completely (typically ~ 150–200 uL) 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 uL) 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.

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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 uL) 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 uL) 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 different colored dye.
7. Repeat until the desired number of antigens have been detected.
8. Once staining is completed, mount tissue sections and image.

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 Fiji (https://fiji.sc/).
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 used. For this reason, control experiments using single-plex staining with and without VectaPlex usage are recommended.

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™.*

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