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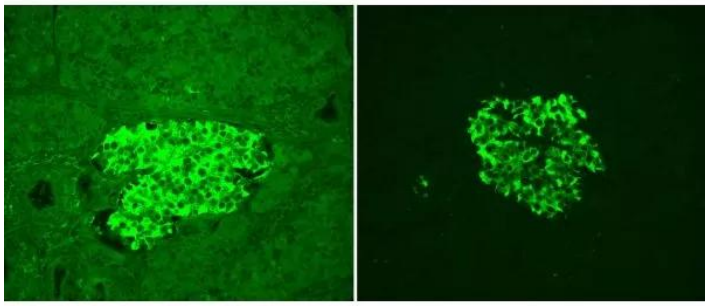
VECTOR TRUEVIEW AUTOFLUORESCENCE QUENCHING KIT

SKU: SP-8400-15



DESCRIPTION

The TrueVIEW Autofluorescence Quenching Kit provides a novel way to diminish unwanted autofluorescence from non-lipofuscin sources and dramatically improve signal-to-noise ratio. TrueVIEW removes unwanted autofluorescence in tissue sections due to aldehyde fixation, the presence of red blood cells, and structural elements such as collagen and elastin. The quenching action of the kit reagents provides a clear, unambiguous, “true view” localization of the target antigen.

This kit offers you:

- Effectivity with problematic tissues such as kidney and spleen
- An easy-to-use, one-step method
- Compatibility with a wide selection of fluorophores
- Compatibility with standard epifluorescence and confocal laser microscopes
- Availability with DAPI counterstain and without.One kit is sufficient to treat approximately 100

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to 150 tissue sections.

SPECIFICATIONS

Blocking Action	Autofluorescence
Format	Concentrate
Unit Size	1 kit
Applications	Immunofluorescence, In situ hybridization

KIT COMPONENTS

What’s in the box?

- TrueVIEW Reagent A, 5 ml
- TrueVIEW Reagent B, 5 ml
- TrueVIEW Reagent C, 5 ml
- VECTASHIELD Vibrance® Antifade Mounting Medium, 2 ml

TECHNICAL INFORMATION

Tissue autofluorescence often occurs with aldehyde fixation or from inherent native tissue components (collagen, elastin, and red blood cells). The extent and intensity of autofluorescence background frequently makes it difficult or impossible to distinguish specific signals in immunofluorescence applications.

Most methods for reduction of tissue autofluorescence act primarily on lipofuscin granules, and are not broadly effective against the most common sources of autofluorescence targeted by TrueVIEW Quencher.

Current methods for reducing autofluorescence primarily include “home brew” concoctions such as sodium borohydride and other ink-based products. These methods are essentially ineffective against aldehyde induced autofluorescence. In contrast, TrueVIEW reagent binds to hydrophilic compounds and effectively quenches endogenous autofluorescence.

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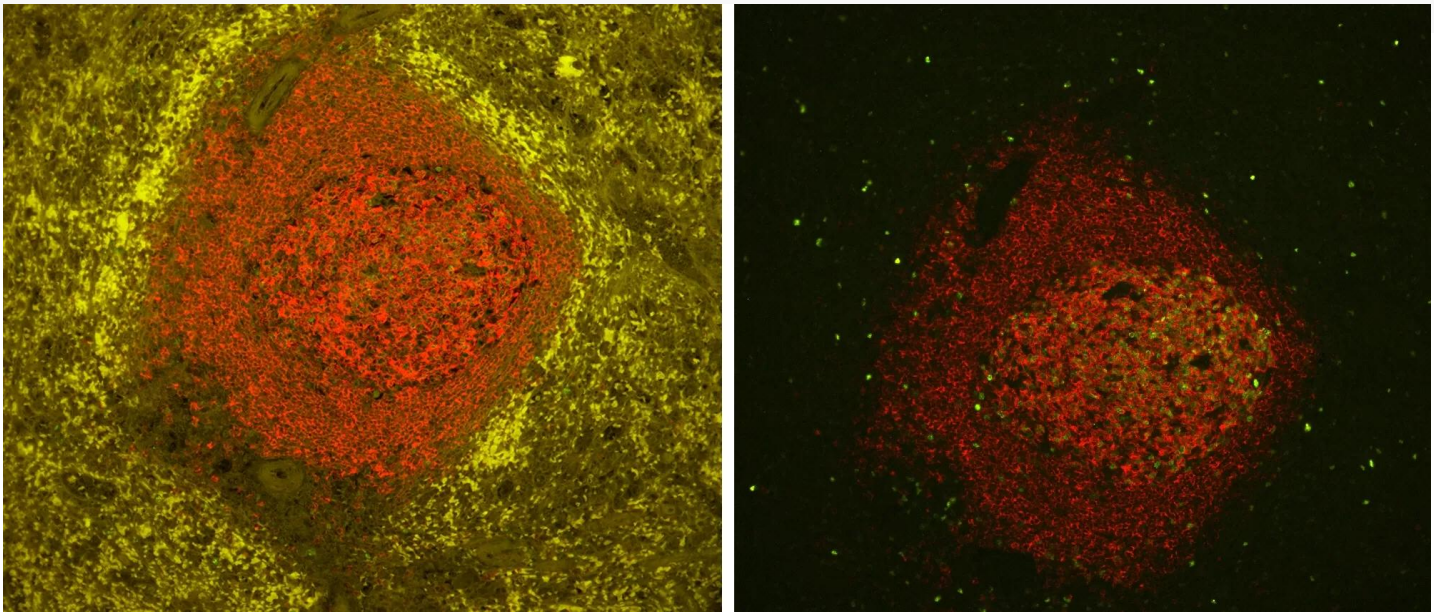

Image showing Spleen FFPE tissue stain, with & without TrueVIEW autofluorescence quencer. Antigen retrieved with Antigen Unmasking Solution. Spleen tissue stained with Mouse Anti-CD20 (red) and Rabbit Anti-Ki67 (green), followed by VectaFluor™ Duet Kit, and mounted in VECTASHIELD Vibrance Mounting Medium with DAPI.

Left Image: No TrueVIEW Quencher treatment – 20x objective, red channel exposed 200ms, green channel 200ms. Note presence of interfering autofluorescence.

Right Image: Cells treated with TrueVIEW Autofluorescence Quencher – 20x objective, red channel exposed 200ms, green channel exposed 800ms. Note absence of autofluorescence.

Human tonsil (FFPE); Section stained for AE1/AE3 using fluorescein label (green). Treated with TrueVIEW and mounted with VECTASHIELD Vibrance Antifade Mounting Medium with DAPI (nuclei blue).

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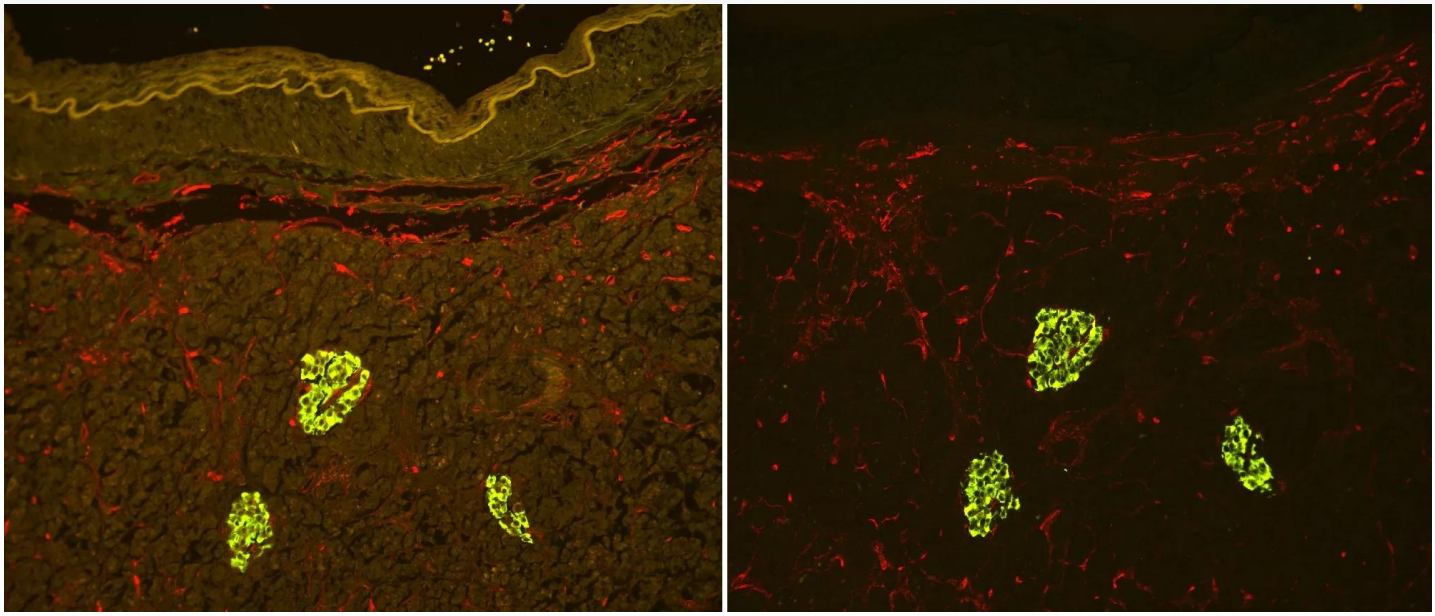

Image showing Pancreas FFPE tissue stain. Antigen retrieved with Antigen Unmasking Solution, stained with Guinea Pig x Insulin (green) and Mouse x END (red), followed by FL-Goat x Guinea Pig IgG + DyLight™ 594-Horse x Mouse IgG. Mounted in VECTASHIELD Vibrance Mounting Medium with DAPI.

Left Image: No TrueVIEW Quencher used, 20x objective, red channel exposed 600ms, green channel 600ms.

Right Image: TrueVIEW Quencher used with 20x objective, red channel exposed 600ms, green channel exposed 600ms.

Easy To Apply

Following completion of the IF staining procedure:

Mode of Action

Following completion of the IF staining procedure:

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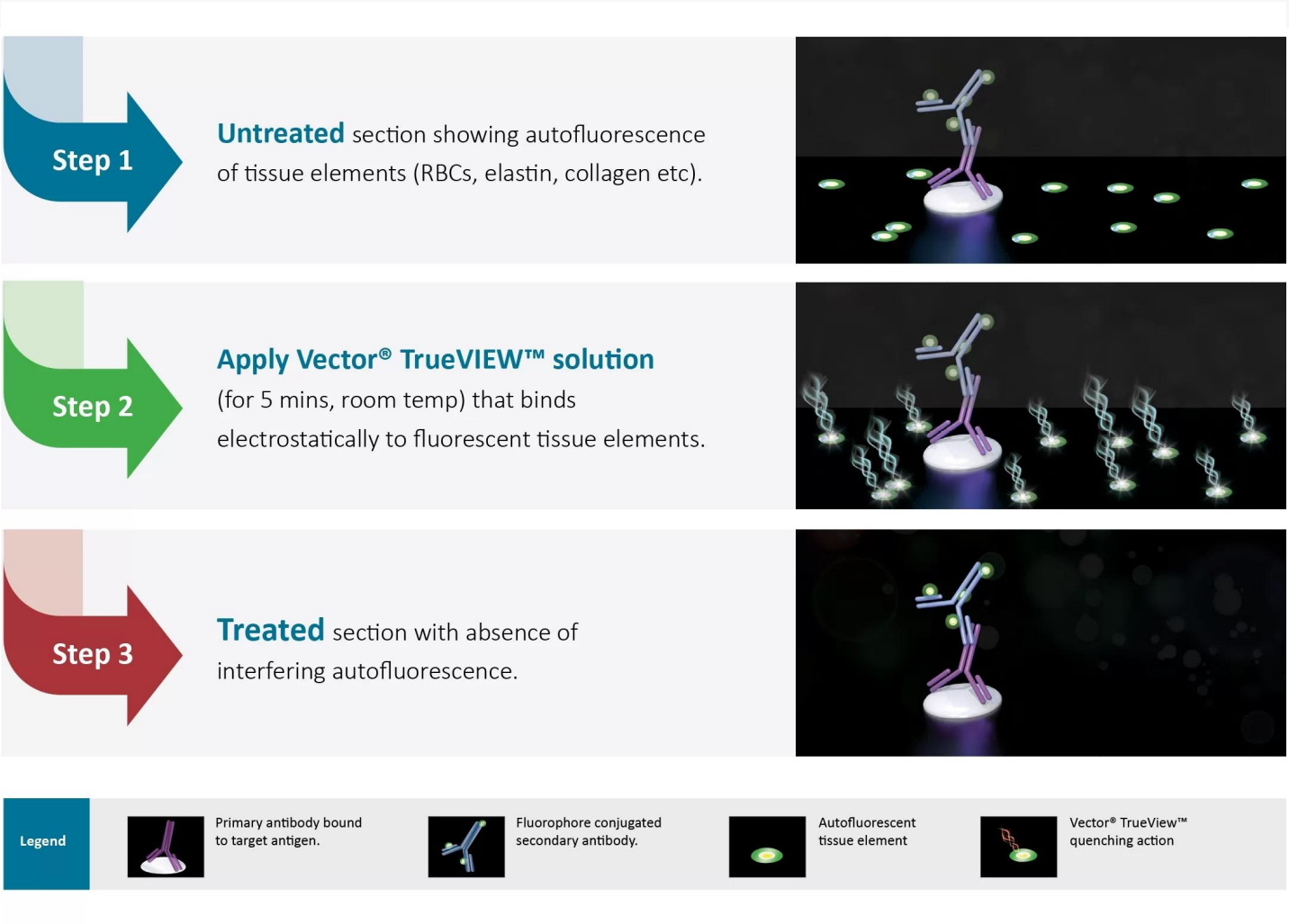

Signal to Noise Optimization

The primary antibody and detection reagents should be optimized (titered) in conjunction with Vector TrueVIEW Quenching Kit to achieve maximum signal to noise.

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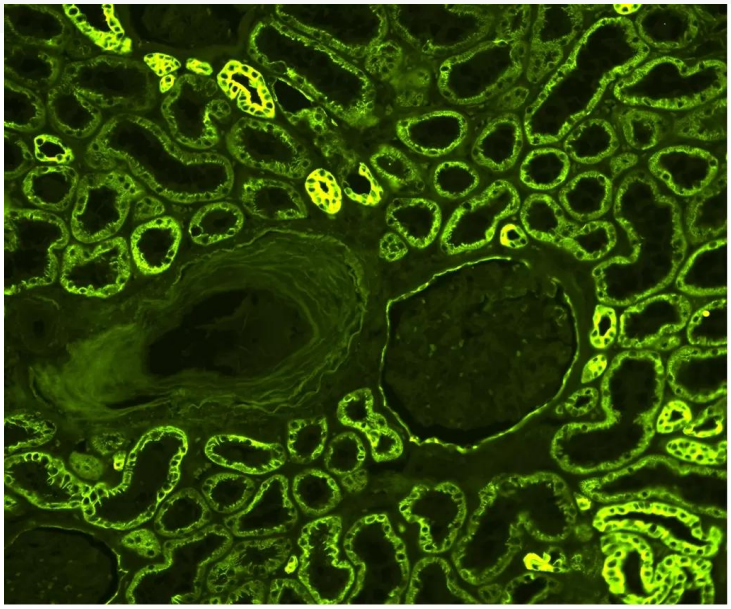
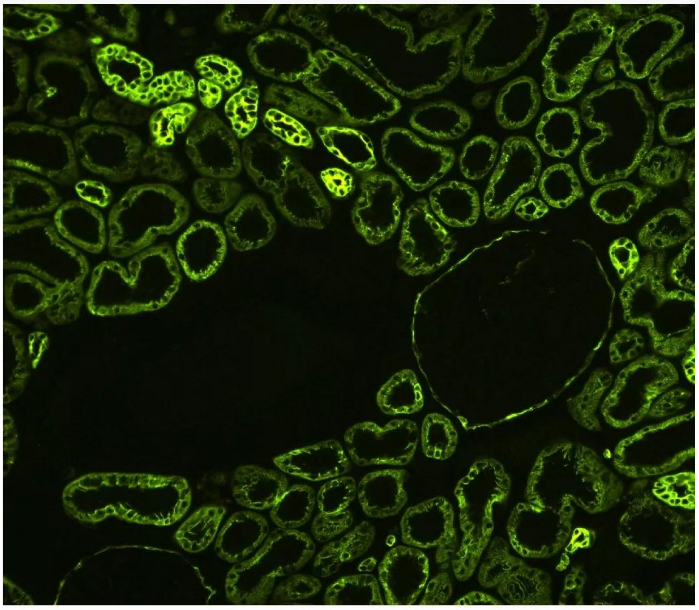
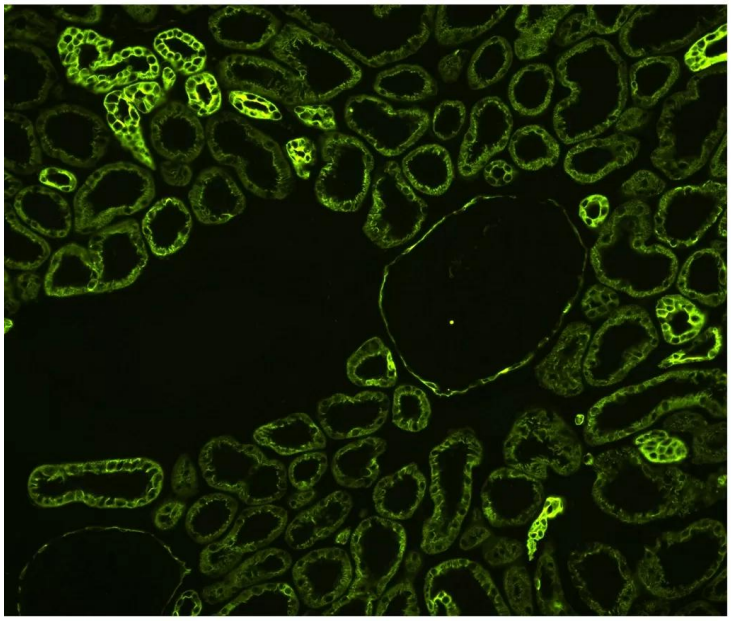


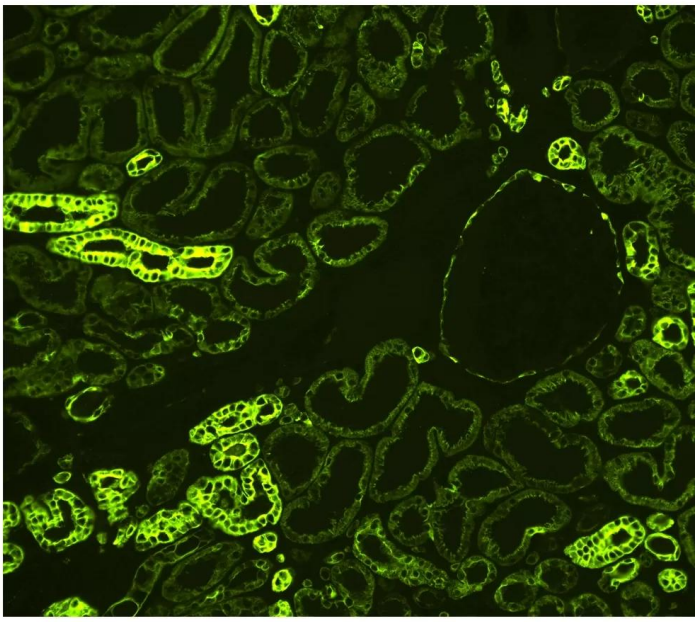
Kidney (FFPE): No TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, **1/100** dilution. 20x. 400 ms exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, **1/25** dilution. 20x. 400 ms exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, **1/50** dilution. 20x. 400 ms exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, **1/100** dilution. 20x. 400 ms exposure.

Optimization of Exposure

Increasing exposure times may be necessary to achieve optimal image acquisition for TrueVIEW

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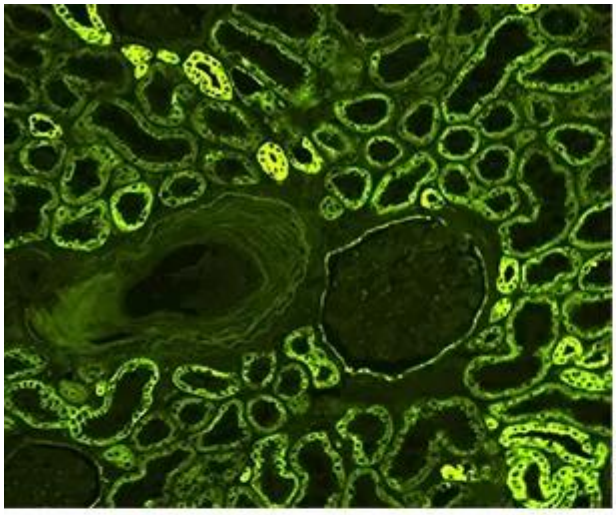
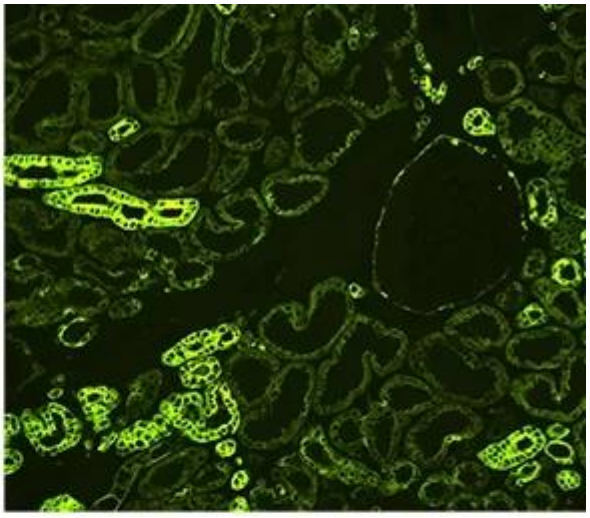
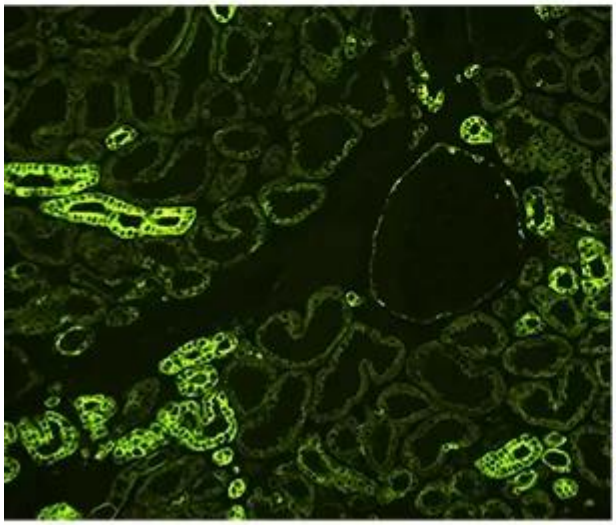
Quencher treated slides.



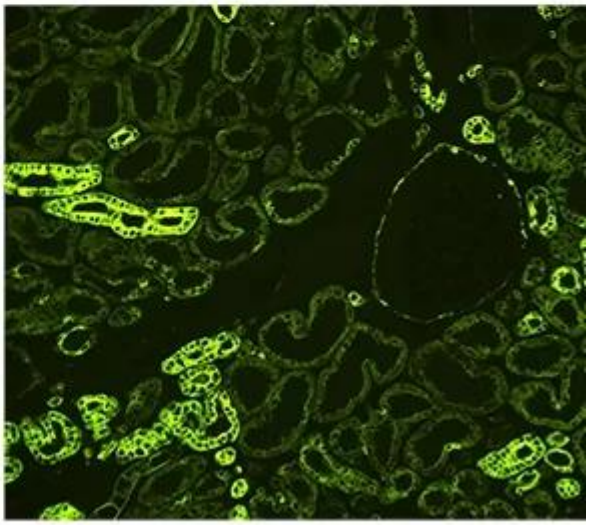
Kidney (FFPE): No TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, 1/100 dilution. 20x. **400 ms** exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, 1/100 dilution. 20x. **400 ms** exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, 1/100 dilution. 20x. **800 ms** exposure.



Kidney (FFPE): With TrueVIEW Quencher, stained with Anti-Pan Cytokeratin, 1/100 dilution. 20x. **1s** exposure.

Comparison Between Vector TrueVIEW Reagent and Other Autofluorescence Reducing Agents

We compared the effectiveness of Vector TrueVIEW quenching action in parallel with other commercially available autofluorescence reducing products and “home brew” reagents, on serial sections of formalin-fixed, paraffin-embedded human pancreas visualized using a standard fluorescein (green) filter. No specific immunofluorescence staining was conducted. The

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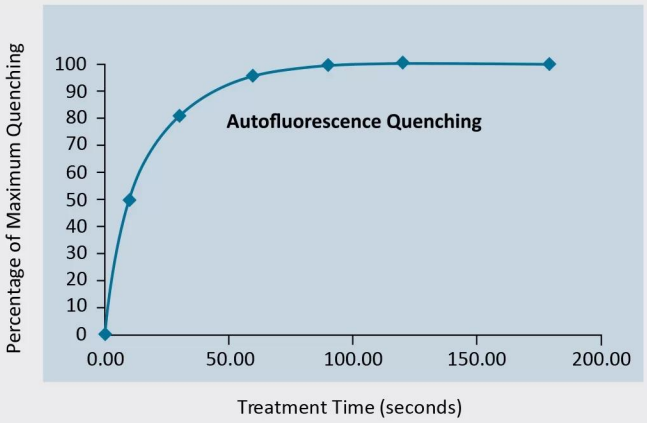
images below highlight our results. All images were acquired under identical conditions (including microscope objective and exposure times).

Rapid Quenching Effect

Quenching of human kidney section autofluoresence at time points after Vector® TrueVIEW™ Treatment.

Note: Maximum quenching of autofluorescence is achieved within 2 minutes of application.



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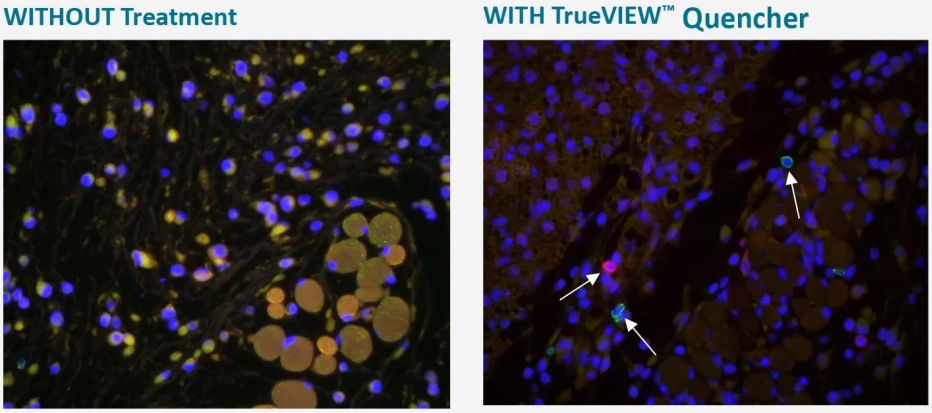
Dr. Kaitlyn Sadtler
Postdoctoral Fellow
MIT, Boston Children's Hospital

"I would definitely use this reagent in the future — it is quick and reliable on multiple tissue types."
— Dr. K. Sadtler



Adjacent mouse skin tissue sections (FFPE) double stained using primary antibodies against CD3 (Pan T cell marker) and B220 (CD45R) antigens with a DAPI (blue) counterstain. Note: interfering background autofluorescence on section without treatment (left image), and essentially total elimination of autofluorescence following treatment with Vector® TrueVIEW™ Quenching Reagent (right image). The arrows on the right image show the "true" actual antigen staining. Courtesy of Dr. Sadtler.



"I tested it after using a tyramide fluorescent detection kit (555) and also tested it with GFP expression models while also comparing this kit to a competitor's quenching solution. The TrueVIEW definitely decreased autofluorescence more than the competition while seeming to have no effect on GFP signal."
— Brian Tabb, MS, HTL, QIHC

Brian Tabb, MS, HTL, QIHC
Immunohistochemistry Scientist
Alizee Pathology

CITATIONS

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DOCUMENTS

- Safety Data Sheet
- TrueVIEW® Application Note
- User Guide
- Download CoA

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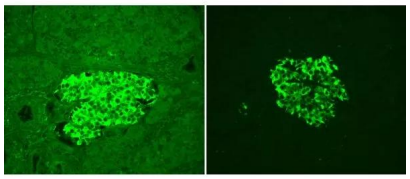
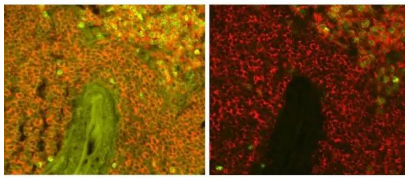
- Datasheet

GALLERY IMAGES







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