

Vector® TrueVIEW®

Autofluorescence Quenching Kit

Cat. No.	SP-8400
Storage	Store reagents in original bottles at 2-8 °C. Avoid storing reagents or working solution in strong direct light.
Description	Vector TrueVIEW Autofluorescence Quenching Kit provides a novel way to remove unwanted fluorescence in tissue sections due to aldehyde fixation, red-blood cells, and structural elements such as collagen and elastin. This unique formulation binds and effectively quenches the autofluorescent elements in even the most problematic tissues, such as kidney, spleen and pancreas. The use of Vector TrueVIEW leads to significant enhancement in overall signal-to-noise in most immunofluorescence assays.

Components

Product Name	Volume
Vector TrueVIEW Reagent A	5 ml
Vector TrueVIEW Reagent B	5 ml
Vector TrueVIEW Reagent C	5 ml
VECTASHIELD Vibrance® Antifade Mounting Medium	2 ml

Important Assay Optimization Process:

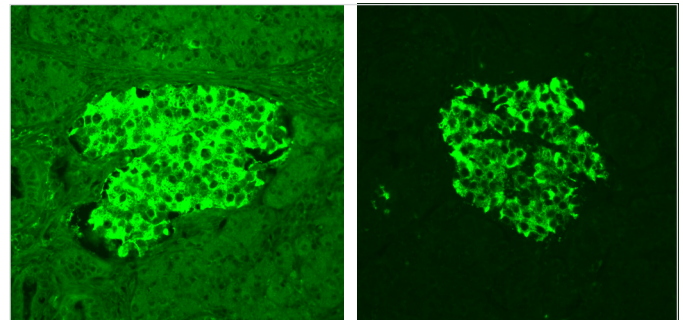
- 1) Determine the extent of autofluorescence with NEGATIVE CONTROL unstained sections (i.e. no detection reagents).
- 2) On adjacent NEGATIVE control unstained sections, observe the quenching effect of the TrueVIEW reagent with a 2-5 min incubation.
- 3) After establishing the control parameters described in 1 & 2 above, optimize the primary antibody dilution to achieve desired signal to noise ratio when using TrueVIEW quenching solution.
- 4) The mounting medium has a significant impact on the performance of this product. Substitution with a different mounting medium other than what is provided in the kit, may dramatically affect the outcome. Vector TrueVIEW quenching reagent has been optimized for use with VECTASHIELD Vibrance (included in kit), and this combination is highly recommended to achieve the best results.

Instructions for Use

A) Reagent Preparation

For each standard tissue section in your assay, you will need approximately 150 µl of Vector TrueVIEW Reagent (i.e., 50 µl Reagent A + 50 µl Reagent B + 50 µl Reagent C).

To prepare Vector TrueVIEW Reagent, a ratio of 1:1:1 of Reagents A, B and C is required. The order of mixing is important.



Human Pancreas (FFPE): Adjacent sections stained for insulin using fluorescein label (green). Mounted with VECTASHIELD Vibrance Antifade Mounting Medium. Note significant reduction of autofluorescence in treated section (right) with the retention of specific staining.

- 1) Add equal volumes of Reagent A and Reagent B in a clean test tube. Mix for 10 seconds.
- 2) Add Reagent C to the mixture (ensuring a 1:1:1 volume ratio) and mix again for 10 seconds.

Vector TrueVIEW Reagent is now ready to use. Once prepared, Vector TrueVIEW Reagent is stable for at least 2 hours at room temperature.

B) Tissue Treatment Procedure

Following completion of the immunofluorescent staining:

- 1) Drain excess buffer from tissue section.
- 2) Add Vector TrueVIEW Reagent to cover tissue section completely (~150 µl); and incubate for 2-5 minutes.
- 3) Wash in PBS buffer for 5 minutes.
- 4) Drain excess buffer from section. Optimal results are obtained if excess buffer is removed around tissue prior to adding mounting medium.
- 5) Dispense VECTASHIELD Vibrance Antifade Mounting Medium onto the tissue section. Coverslip and allow VECTASHIELD Vibrance Antifade Mounting Medium to disperse over the entire section.
- 6) Slides can be visualized immediately after mounting, but the coverslip will not be immobilized until mounting media is cured at room temperature for 1-2 hours.
- 7) For optimal results, slides should be evaluated within 48 hours of mounting.

C) Nuclear Counterstaining

Nuclear counterstaining can be performed after TrueVIEW treatment. Counterstain concentration may need to be increased to achieve optimal signal to noise. For DAPI staining, we recommend using DAPI at 5 µg/ml in PBS buffer for 10 minutes, and then mounting with the included VECTASHIELD Vibrance Antifade Mounting Medium.