VECTOR LABORATORIES

part of Maravai LifeSciences

USER GUIDE

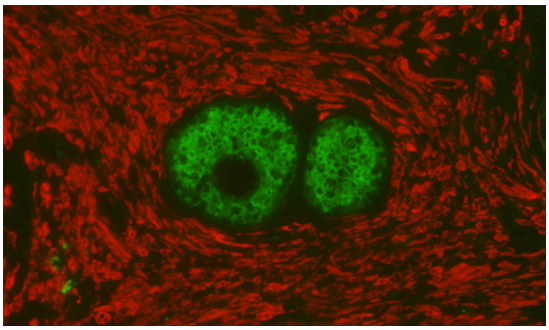
VectaFluor™ Duet Double Labeling Kit

DyLight® 488 Anti-Rabbit IgG, DyLight® 594 Anti-Mouse IgG

Cat. No.: DK-8818

Storage: 2-8 °C

Instructions for immunofluorescent staining.



Prostate: PSA (rb) and Smooth Muscle Actin (m) detected simultaneously with VectaFluor Duet IF Double Labeling Kit, DyLight 488 Anti-Rabbit (green)/DyLight 594 Anti-Mouse (red). Mounted in VECTASHIELD® HardSet™ Antifade Mounting Medium.

DESCRIPTION

VectaFluor Duet Double Labeling Kit offers maximum convenience to achieve double label immunofluorescence staining. This kit can detect mouse and rabbit primary antibodies with green and red DyLight fluorescent dyes in one step and is intended for use on non-rodent specimens.

KIT COMPONENTS

Product Name	Volume
Normal Horse Serum, 2.5%	15 ml
VectaFluor Duet Reagent [DyLight 488 Anti-Rabbit IgG (Green), DyLight 594 Anti-Mouse IgG (Red)]	15 ml

The VectaFluor Double Labeling Kit will stain approximately 150 sections based on 100 µl per section.

STORAGE:

- Store reagents in original bottles at 2-8 °C
- Do not freeze.

PREPARATION OF WORKING SOLUTIONS

VectaFluor Double Labeling Kit reagents are ready-to-use — no mixing or titering is necessary to obtain optimal staining.

The staining procedure should be performed at room temperature (20-25°C). VectaFluor Double Labeling Kit reagents should be equilibrated to room temperature for optimal performance.

A number of different wash buffers can be used. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% saline (PBS). 0.1% Tween 20 detergent may be added to the wash buffer and is especially recommended for use with automated stainers.

STAINING PROCEDURE

1. For paraffin sections, deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.

   For frozen sections or cell preparations fix with acetone or an appropriate fixative for the antigen under study, if required.

2. If antigen unmasking is required, perform this procedure using a Vector® Antigen Unmasking Solution, Citrate-based (H-3300) or Tris-based (H-3301).

3. Wash in buffer for 5 minutes.

4. Incubate for 20 minutes with 2.5% Normal Horse Serum (or blocking solution of choice).

5. Tip off excess serum from sections.

6. Incubate with mouse primary antibody diluted in an appropriate diluent.

7. Wash in buffer for 5 minutes.

8. Incubate with rabbit primary antibody diluted in an appropriate diluent.

9. Wash in buffer for 2 x 5 minutes.

10. Incubate for 30 minutes with VectaFluor Duet Reagent.

11. Wash for 2 x 5 minutes in buffer.

12. Mount in a media suitable for fluorescence, such as one of the VECTASHIELD Antifade Mounting Media.

Detailed product listing, specifications, protocols and additional information are available on our website: **vectorlabs.com**

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