



VectaFluor™ Duet Double Labeling Kit

DyLight® 594 Anti-Rabbit IgG (Red)
DyLight® 488 Anti-Mouse IgG (Green)

Cat. No. DK-8828

Introduction

VectaFluor™ Duet 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**. DyLight® dyes offer several advantages such as brighter fluorescence, greater photostability, and pH independence. Our affinity purified, extensively cross-adsorbed secondary antibodies are conjugated to DyLight® dyes in a manner that ensures maximum degree of labeling without compromising antibody affinity or specificity. VectaFluor™ Duet is compatible with fluorescence staining of cells and tissues.

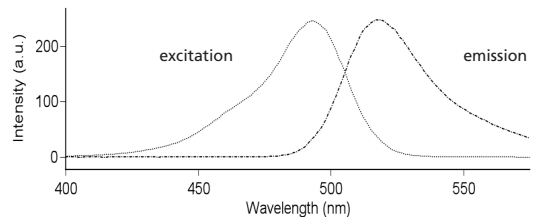
VectaFluor™ Duet Kit contains 15 ml pre-diluted, stabilized, RTU Duet Detection Reagent along with 15 ml RTU 2.5% normal horse serum for blocking.

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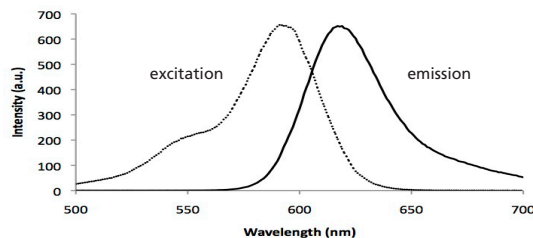
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DyLight® 488 conjugate Spectral Properties



Excitation maximum is at 493 nm. Emission maximum is at 518 nm.

DyLight® 594 conjugate Spectral Properties



Excitation maximum is at 592 nm. Emission maximum is at 617 nm.

COMPONENTS

Reagents supplied:

- 15 ml 2.5% Normal Horse Serum (ready-to-use) for blocking
- 15 ml VectaFluor™ Duet Reagent [DyLight® 594 Anti-Rabbit IgG and DyLight® 488 Anti-Mouse IgG cocktail (rabbit-red, mouse-green,)] made in horse, ready-to-use in 5% NHS.

The VectaFluor™ Duet Kit will stain approximately 150 sections based on 100 µl per section.

VectaFluor™ Duet Kit reagents are designed for laboratory use only.

Storage:

Store the VectaFluor™ Duet Kit reagents at 2-8 °C (do not freeze).

Reagents not supplied:

- Buffer
- Primary Antibody
- Primary Antibody Diluent

VectaFluor™ Duet Antibody Reagent

VectaFluor™ Duet Reagents are ready-to-use. No mixing or titering of the VectaFluor™ Duet Reagent is necessary to obtain optimal staining. Dilution of this reagent or changes in the suggested incubation time may affect performance. The reagents are supplied in convenient dropper bottles. (To remove the drop dispenser tip, press the tip laterally with thumb until it snaps off).

The staining procedure should be performed at room temperature (20-25°C). The VectaFluor™ Duet Kit reagents should be equilibrated to room temperature for optimal performance. Slides should be placed in a humidified chamber during the incubation period.

A number of different buffers can be used with the VectaFluor™ Duet Kit. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% sodium chloride (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, pH 6.0 (H-3300) or Tris-based, pH 9.0 (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). Tip off.
5. Incubate with mouse primary antibody diluted in an appropriate diluent. (See Note 1, 2).
6. Wash in buffer for 5 minutes.
7. Incubate with rabbit primary diluted in an appropriate diluent. (See Note 1,2)

8. Wash in buffer for 2 x 5 minutes.
9. Incubate for 30 minutes with **VectaFluor™ Duet Reagent**.
10. Wash for 2 x 5 minutes in buffer.
11. Mount in a media suitable for fluorescence, such as one of the VECTASHIELD® Mounting Media. See product listing.

NOTES:

1. To avoid adsorption of the antibody to the plastic or glass container in which the final dilution is made, the primary antibody may be diluted in buffers containing diluted (2.5%) normal horse serum (S-2000 or S-2012). Alternatively, immunohistochemical grade Bovine Serum Albumin (SP-5050) can be used. Other grades of BSA can contain undesired impurities.
2. If the appropriate controls are included, primary antibodies can be combined and incubated as a 1-Step cocktail.
3. For thicker sections, longer incubation times may be required for optimal staining. Appropriate control slides should be run in parallel if incubation times are altered.
4. Aldehyde-fixed tissue (e.g. formalin) and certain endogenous cellular tissue elements may be autofluorescent. This may make interpretation of a specific fluorescein signal difficult. Use proper controls to determine if autofluorescence is a problem.
5. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining. It is recommended that solutions be prepared with glass distilled water.
6. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.

VectaFluor™ Kits and Staining Systems:

VectaFluor™ Double Labeling Kits

VectaFluor™ Duet Double Labeling Kit
DyLight® 488 Anti-Rabbit IgG
DyLight® 594 Anti-Mouse IgG 1 Kit DK-8818

VectaFluor™ Duo Double Labeling Kit
DyLight® 594 Anti-Rabbit IgG
DyLight® 488 Anti-Mouse IgG 1 Kit DK-8828

VectaFluor™ Amplified Fluorescent Staining Systems

VectaFluor™ Excel Anti-Rabbit IgG Kit
(DyLight® 488) 1 Kit DK-1488

VectaFluor™ Excel Anti-Rabbit IgG Kit
(DyLight® 594) 1 Kit DK-1594

VectaFluor™ Excel Anti-Mouse IgG Kit
(DyLight® 488) 1 Kit DK-2488

VectaFluor™ Excel Anti-Mouse IgG Kit
(DyLight® 594) 1 Kit DK-2594

VectaFluor™ Reagent Kits

VectaFluor™ Anti-Rabbit IgG Kit
(DyLight® 488) 1 Kit DI-1788

VectaFluor™ Anti-Rabbit IgG Kit
(DyLight® 594) 1 Kit DI-1794

VectaFluor™ Anti-Mouse IgG Kit
(DyLight® 488) 1 Kit DI-2788

VectaFluor™ Anti-Mouse IgG Kit
(DyLight® 594) 1 Kit DI-2794

VectaFluor™ Anti-Goat IgG Kit
(DyLight® 488) 1 Kit DI-3788

VectaFluor™ Anti-Goat IgG Kit
(DyLight® 594) 1 Kit DI-3794

Additional Reagents:

VECTASHIELD® Mounting Medium		
no counterstain	10 ml	H-1000
with DAPI	10 ml	H-1200
with Propidium Iodide	10 ml	H-1300

VECTASHIELD® mounting media provide strong initial fluorescence, retard photobleaching during illumination, preserve the fluorescent signal on storage, and has an ideal refractive index.

VECTASHIELD® Hard+Set™ Mounting Medium		
no counterstain	10 ml	H-1400
with DAPI	10 ml	H-1500
with TRITC-Phalloidin		

VECTASHIELD® Hard+Set™ mounting media have all the properties of VECTASHIELD® but in a hardening formula.

Animal-Free Block & Diluent (RTU) S-5035 500 mg R.T.U. Animal Free Block and Diluent is a ready-to-use solution that can be used both as an antibody diluent and a blocking reagent to diminish background due to non-specific binding in immunohistochemical applications. This product contains no animal-derived proteins and can be used as an alternative to sera, BSA, casein or non-fat dry milk in situations where these or other traditional blocking agents are not effective.

BSA S-5050 500 mg
This ultra pure grade of bovine serum albumin can be used as a diluent or a blocking agent. It is free of impurities present in other grades of BSA.

Heat-treated, ultrafiltered normal serum

Normal Horse Serum	S-2000	20 ml
2.5 % Normal Horse Serum	S-2012	50 ml

Sera are obtained from healthy adult animals, heat treated at 56 °C for 2 hours, incubated at 4 °C to precipitate cryoglobulins, ultracentrifuged and ultrafiltered through a 0.45µm filter.

Control Antibodies

Rabbit IgG Control Antibody	1 mg	I-1000
Mouse IgG Control Antibody	1 mg	I-2000
Goat IgG Control Antibody	1 mg	I-5000

VECTABOND™ Reagent 7 ml SP-1800

VECTABOND™ Reagent is designed to significantly increase adherence of both frozen and paraffin embedded tissue sections to glass slides during standard immunohistochemical procedures, or under harsh conditions such as required for high temperature antigen unmasking techniques or *in situ* hybridization. This product chemically modifies the glass to form a highly adherent surface. VECTABOND™ Reagent is provided as a 50x concentrated stock sufficient for treating at least 500 slides.

ImmEdge™ Pen 2-pen set H-4000
The ImmEdge™ Pen provides a pale blue, hydrophobic, heat-stable barrier that keeps reagents localized to tissue sections.

ImmPrint™ Histology Pen 5-pen set H-6100
This black permanent marking pen is resistant to most organic solvents encountered in histological applications and is designed to write on glass slides, tissue cassettes, and most hard surfaces.

Antigen Unmasking Solution

Citrate-based, 6.0 pH	250 ml	H-3300
Tris-based, 9.0 pH	250 ml	H-3301

These formulas are highly effective at revealing antigens in formalin-fixed, paraffin-embedded tissue sections using a high temperature treatment procedure. Antigen Unmasking Solutions are supplied as 100x concentrated stock sufficient to prepare 25 liters of working solution.

Trademarks: Vector, VectaFluor, VECTABOND, VECTASHIELD, ImmEdge, ImmPrint, are trademarks of Vector Laboratories. DyLight is a trademark of Thermo Fisher.

Detailed product listings, specifications and protocols are available on our website. A complete catalog listing is also available upon request.

Visit our website: www.vectorlabs.com