



VectaFluor™ *Excel*

R.T.U. Antibody Kit

DyLight® 594

Anti-Mouse Ig

Cat. No. DK-2594

Introduction

VectaFluor™ *Excel* Amplified Fluorescent Staining System offers a non-biotin amplification method for fluorescence applications. This system uses a proprietary, ready-to-use (R.T.U.) Amplifier Antibody, followed by a ready-to-use (R.T.U.) VectaFluor™ DyLight® dye-labeled detection system.

All the reagents in the VectaFluor™ *Excel* Kit are affinity purified and extensively cross-adsorbed to ensure high sensitivity and low background. In addition, the VectaFluor™ Reagents are conjugated to the DyLight® dyes using technology that ensures maximum labeling without compromising antibody affinity or specificity. DyLight® dyes offer several advantages such as brighter fluorescence, greater photostability, and pH independence.

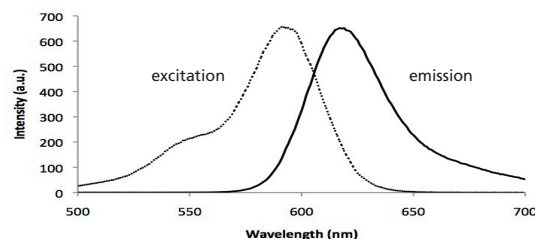
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DyLight® 594 conjugate Spectral Properties



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 Amplifier Antibody (goat anti-mouse Ig, ready-to-use)
- 15 ml VectaFluor™ DyLight® Dye-Labeled Anti-Goat IgG (made in horse, ready-to-use)

This kit is not recommended for multiple-labeling fluorescent applications.

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

Unless labeled otherwise, VectaFluor™ *Excel* Antibody Kit reagents are designed for laboratory use only.

Storage:

Store VectaFluor™ *Excel* Antibody Kit reagents at 2-8 °C (do not freeze).

Reagents not supplied:

- Buffer
- Primary Antibody
- Primary Antibody Diluent

VectaFluor™ *Excel* Antibody Kit

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

The staining procedure should be performed at room temperature (20-25 °C). The VectaFluor™ *Excel* Antibody 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 wash buffers can be used with the VectaFluor™ *Excel* Antibody Kit reagents. 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, if necessary, fix with acetone or an appropriate fixative for the antigen under study.

2. If antigen unmasking is required, perform this procedure using a Vector® Antigen Unmasking Solution, Citrate-based (H-3300) or High pH-based (H-3301).
3. Wash in buffer for 5 minutes.
4. Incubate sections for 20 minutes with ready-to-use 2.5% Normal Horse Serum. Tip off.
5. Incubate with mouse primary antibody diluted in an appropriate diluent. See Note 4.
6. Wash in buffer for 5 minutes.
7. Incubate for 15 minutes with Amplifier Antibody.
8. Wash in buffer for 5 minutes.
9. Incubate for 30 minutes with VectaFluor™ 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 on reverse side.

NOTES:

1. 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.
2. 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. References for reducing autofluorescence are available upon request.
3. 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.
4. 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 a buffer containing diluted (2.5%) normal horse serum (Cat. No. S-2000 or S-2012). Do not substitute serum from other species to avoid background due to cross-reactivity.

Alternatively, 0.1% immunohistochemical grade Bovine Serum Albumin (Cat. No. SP-5050). Only immunohistochemical grade BSA should be used, as other preparations can contain undesired impurities.

5. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (Cat. No. SP-1800), a non-protein tissue section adhesive.

Additional VectaFluor™ Amplified Fluorescent Staining Systems:

VectaFluor™ Exoel Anti-Rabbit Ig Kit
(DyLight® 594) 1 Kit DK-1594

VectaFluor™ Exoel Anti-Mouse Ig Kit
(DyLight® 488) 1 Kit DK-2488

VectaFluor™ Exoel Anti-Rabbit Ig Kit
(DyLight® 488) 1 Kit DK-1488

Additional VectaFluor™ Reagent Kits:

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

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

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

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

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

VectaFluor™ Anti-Goat Ig 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® Hard+Set™ Mounting Medium

no counterstain	10 ml	H-1400
with DAPI	10 ml	H-1500

VECTASHIELD® Mounting Medium provides strong initial fluorescence, retards photobleaching during illumination, preserves the fluorescent signal on storage, and has an ideal refractive index.

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

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. 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	250 ml	H-3300
High 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.

Serum

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.

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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