

Immunofluorescence Selection Guide

Follow the simple steps below to choose the most appropriate labeling and detection solution for your experiment.

1 Choose Primary Antibody

- Specific for antigen of interest
- Consider tissue species and preparation (fixation)
- Consider antigen retrieval requirements



VECTABOND® Reagent
(Tissue Section Adhesive)

Blocking Reagents

- Choices determined by the options selected in Steps 1-2
- Streptavidin/Biotin Blocking Kit (if using biotin/streptavidin system)
- Avidin/Biotin Blocking Kit
- Normal Sera (from the species of secondary antibody)
- M.O.M.® Mouse Ig Blocking Reagent
- R.T.U. Animal-Free Blocker® and Diluent
- BSA
- Casein Solution

2 Choose Secondary Antibody and Tertiary Detection System

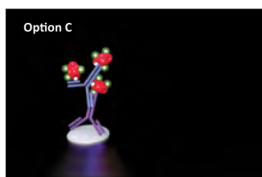
- Choose fluorophore based on wavelengths available in microscope
- Fluorophore-conjugated secondary antibody or biotinylated secondary antibody
- Consider sensitivity requirements
- Consider species of primary antibody
- Consider tissue species



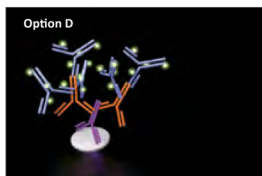
Option A



Option B



Option C



Option D

One Step

- Single-label.
Fast. Convenient.
- Fluorophore-conjugated secondary antibodies
 - VectaFluor™ R.T.U. DyLight® Labeled Secondary Antibodies



One Step

- Dual-label two-antigen detection.
Fast.
- VectaFluor Duet Immunofluorescence Double Labeling Kits



Two Step

- Biotin-based.
- Biotinylated secondary antibody and fluorescent avidin or streptavidin Fluorochrome conjugated.
 - See Step 3 for additional amplifications



Two Step

- Highest sensitivity. Non-biotin based.
- VectaFluor Excel Amplified Fluorescent Staining System (Amplifier Antibody + fluorescent tertiary antibody)

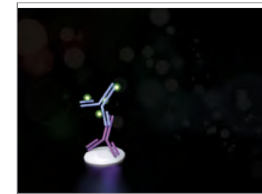
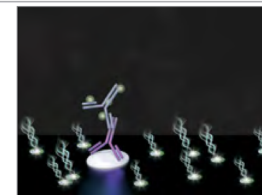
3 Choose Signal Amplification with Biotin-based Systems (Option C)

- Multiple rounds of amplification possible (with biotin-based systems)



4 Reduce Autofluorescence from Aldehyde Fixation

- TrueVIEW™ Autofluorescence Quenching Kit



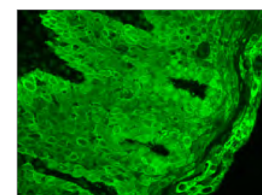
5 Choose Mounting Media with or without a Counterstain

- VECTASHIELD® antifade mounting media, with or without counterstain



6 Visualize

- Fluorescence microscope
- View using appropriate excitation/emission filters



Tonsil (FFPE): Cytokeratin detected with VectaFluor Anti-Rabbit IgG, DyLight 488 (green). Autofluorescence quenched with TrueVIEW Quenching and mounted with VECTASHIELD Vibrance.

Legend



Primary antibody



Fluorophore-conjugated secondary antibody



Amplifier antibody



Biotinylated anti-avidin / streptavidin



Fluorophore



Biotin



Fluorophore-conjugated avidin / streptavidin



TrueVIEW Quenching Action

Available in Canada from...

MJS
BioLynx
INC.

1-888-593-5969
www.biolynx.ca • tech@biolynx.ca

Ordering Information

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Tissue Preparation	
Product	Cat. No.
VECTABOND Reagent (Tissue Section Adhesive)	SP-1800
ImmPrint Histology Pen	H-6100
ImmEdge Hydrophobic Barrier Pen	H-4000

Antigen Retrieval	
Product	Cat. No.
Antigen Unmasking Solution Citrate-based (100X) (pH 6.0)	H-3300
Antigen Unmasking Solution Tris-based (100X) (pH 9.0)	H-3301

Quench/Block	
Product	Cat. No.
TrueVIEW Autofluorescence Quenching Kit (without Counterstain)	SP-8400
TrueVIEW Autofluorescence Quenching Kit (with DAPI Counterstain)	SP-8500
BLOXALL® Endogenous HRP and AP Blocking Solution	SP-6000
Avidin/Biotin Blocking Kit	SP-2001
Streptavidin/Biotin Blocking Kit	SP-2002
Normal Goat Serum	S-1000
Normal Horse Serum	S-2000
Normal Rabbit Serum	S-5000
2.5% Normal Horse Serum	S-2012
Bovine Serum Albumin (BSA)	SP-5050
10x Casein Solution	SP-5020
R.T.U. Animal-Free Blocker & Diluent	SP-5035
M.O.M. Mouse Ig Blocking Reagent	MKB-2213
Carbo-Free™ Blocking Solution	SP-5040

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Fluorochrome-Labeled Secondary Antibodies									
Product	AMCA	Fluorescein	DyLight 488	DyLight 549	DyLight 594	DyLight 649	Cy3®	Cy5®	
Anti-Mouse IgG (H+L), made in horse		FI-2000	DI-2488	DI-2549	DI-2594	DI-2649	CY-2300	CY-2500	
Anti-Mouse IgG (H+L), rat adsorbed, made in horse		FI-2001							
Anti-Mouse IgM, mu chain specific, made in goat		FI-2020							
Anti-Rabbit IgG (H+L), made in horse			DI-1088		DI-1094				
Anti-Rabbit IgG (H+L), made in goat		FI-1000	DI-1488	DI-1549	DI-1594	DI-1649	CY-1300	CY-1500	
Anti-Rat IgG (H+L), made in rabbit		FI-4000							
Anti-Rat IgG (H+L), mouse adsorbed, made in rabbit		FI-4001							
Anti-Rat IgG, made in goat							CY-4300	CY-4500	
Anti-Goat IgG (H+L), made in horse			DI-3088		DI-3094				
Anti-Goat IgG (H+L), made in rabbit	CI-5000	FI-5000							
Anti-Sheep IgG (H+L), made in rabbit		FI-6000							
VectaFluor™ Anti-Rabbit IgG, made in horse			DI-1788		DI-1794				
VectaFluor™ Anti-Mouse IgG, made in horse					DI-2794				
Anti-Human Fluorochrome-Labeled Secondary Antibodies									
Anti-Human IgG (H+L), made in goat		FI-3000							
Anti-Human IgE, epsilon chain specific, made in goat		FI-3040							
Anti-Human IgG, gamma chain specific, made in goat		FI-3080							
Anti-Human IgM, mu chain specific, made in goat		FI-3020							
Anti-Human Kappa Chain, made in goat	CI-3060	FI-3060							
Anti-Human Lambda Chain, made in goat	CI-3070	FI-3070							

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Tertiary Reagents									
Product	Biotin	AMCA	Fluorescein	DyLight 488	DyLight 549	DyLight 594	DyLight 649	Cy3	Cy5
VectaFluor Excel Amplified Anti-Rabbit IgG Kit				DK-1488		DK-1594			
VectaFluor Excel Amplified Anti-Mouse IgG Kit				DK-2488		DK-2594			
Streptavidin		SA-5008	SA-5001	SA-5488	SA-5549	SA-5594	SA-5649	SA-1300	SA-1500
Avidin			A-2001						
Avidin DCS			A-2011						
Anti-Streptavidin	BA-0500			SP-4488	SP-4549				
Anti-Avidin	BA-0300								

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Secondary Antibody Kits	
Product	Cat. No.
VectaFluor Duet Kits	
VectaFluor Duet IF Double Labeling Kit - DyLight 488 Anti-Rabbit (green) - DyLight 594 Anti-Mouse (red)	DK-8818
VectaFluor Duet IF Double Labeling Kit - DyLight 594 Anti-Rabbit (red) - DyLight 488 Anti-Mouse (green)	DK-8828
M.O.M. (Mouse on Mouse) Kits	
M.O.M. Basic Kit	BMK-2202
M.O.M. Fluorescein Kit	FMK-2201

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Counterstain/Mount	
Product	Cat. No.
Non-Hardening Formula	
VECTASHIELD Antifade Mounting Medium	H-1000
VECTASHIELD Antifade Mounting Medium with DAPI	H-1200
VECTASHIELD Antifade Mounting Medium with PI	H-1300
Hardening Formula	
VECTASHIELD HardSet™ Antifade Mounting Medium	H-1400
VECTASHIELD HardSet Antifade Mounting Medium with DAPI	H-1500
VECTASHIELD HardSet Antifade Mounting Medium with TRITC-Phalloidin	H-1600
VECTASHIELD Vibrance™ Antifade Mounting Medium	H-1700
VECTASHIELD Vibrance Antifade Mounting Medium with DAPI	H-1800