

# 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



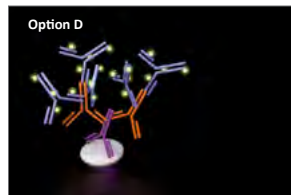
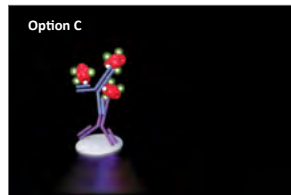
VECTABOND™ Reagent  
(Tissue Section Adhesive)

### Blocking Reagents

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

## 2 Choose Secondary Antibody and Tertiary Detection System

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



SECONDARY DETECTION SYSTEMS

### One Step

- Single-label.  
Fast. Convenient.
- Fluorochrome-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

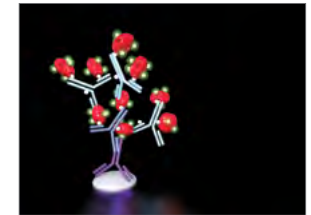


### Two Step

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

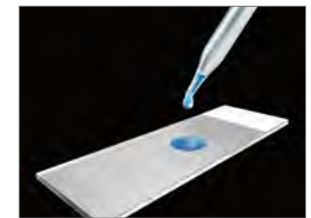
## 3 Choose Signal Amplification with Biotin-based Systems (Option C)

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



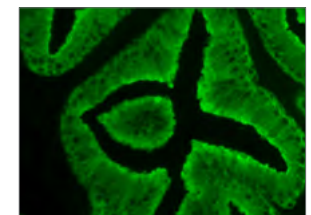
## 4 Choose Mounting Media with or without a Counterstain

- VECTASHIELD® antifade mounting media, with or without counterstain



## 5 Visualize

- Fluorescence microscope
- View using appropriate excitation/emission filters



Prostate: Anti-PSA (goat), VectaFluor™ DyLight® 488 Anti-Goat IgG. Mounted in VECTASHIELD® HardSet™ Mounting Medium.

| Legend |  |  |  |  |  |
|--------|--|--|--|--|--|
|        | Primary antibody                         |  | Fluorochrome-labeled secondary antibody  |  | Amplifier antibody                       |
|        | Biotinylated anti-avidin/streptavidin    |  | Fluorochrome                             |  | Biotin                                   |
|        | Fluorochrome-labeled avidin/streptavidin |  | Fluorochrome-labeled avidin/streptavidin |  | Fluorochrome-labeled avidin/streptavidin |

# Ordering Information

1

| Tissue Preparation                           |                         |
|--|-------------------------|
| Product                                      | Cat. No.                |
| VECTABOND™ Reagent (Tissue Section Adhesive) | <a href="#">SP-1800</a> |
| ImmPrint™ Histology Pen                      | <a href="#">H-6100</a>  |
| ImmEdge™ Hydrophobic Barrier Pen             | <a href="#">H-4000</a>  |

| Antigen Retrieval  |                        |
|--|------------------------|
| Product  | Cat. No.               |
| Antigen Unmasking Solution Citrate-based (100X) (pH 6.0) | <a href="#">H-3300</a> |
| Antigen Unmasking Solution Tris-based (100X) (pH 9.0)    | <a href="#">H-3301</a> |

| Quench/Block                                     |                          |
|--|--------------------------|
| Product  | Cat. No.                 |
| BLOXALL™ Endogenous HRP and AP Blocking Solution | <a href="#">SP-6000</a>  |
| Avidin/Biotin Blocking Kit                       | <a href="#">SP-2001</a>  |
| Streptavidin/Biotin Blocking Kit                 | <a href="#">SP-2002</a>  |
| Normal Goat Serum                                | <a href="#">S-1000</a>   |
| Normal Horse Serum                               | <a href="#">S-2000</a>   |
| Normal Rabbit Serum                              | <a href="#">S-5000</a>   |
| 2.5% Normal Horse Serum                          | <a href="#">S-2012</a>   |
| Bovine Serum Albumin (BSA)                       | <a href="#">SP-5050</a>  |
| 10x Casein Solution                              | <a href="#">SP-5020</a>  |
| R.T.U. Animal-Free Block and Diluent             | <a href="#">SP-5035</a>  |
| M.O.M.™ Mouse Ig Blocking Reagent                | <a href="#">MKB-2213</a> |
| Carbo-Free™ Blocking Solution                    | <a href="#">SP-5040</a>  |

2

| Fluochrome-Labeled Secondary Antibodies            |                         |                         |                         |                         |                         |                         |                         |                         |  |
|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--|
| Product  | AMCA                    | Fluorescein             | DyLight® 488            | DyLight® 549            | DyLight® 594            | DyLight® 649            | Cy3                     | Cy5                     |  |
| Anti-Mouse IgG (H+L), made in horse                | <a href="#">CI-2000</a> | <a href="#">FI-2000</a> | <a href="#">DI-2488</a> | <a href="#">DI-2549</a> | <a href="#">DI-2594</a> | <a href="#">DI-2649</a> | <a href="#">CY-2300</a> | <a href="#">CY-2500</a> |  |
| Anti-Mouse IgG (H+L), rat adsorbed, made in horse  |                         | <a href="#">FI-2001</a> |                         |                         |                         |                         |                         |                         |  |
| Anti-Mouse IgM, mu chain specific, made in goat    |                         | <a href="#">FI-2020</a> |                         |                         |                         |                         |                         |                         |  |
| Anti-Rabbit IgG (H+L), made in horse               |                         |                         | <a href="#">DI-1088</a> | <a href="#">DI-1094</a> |                         |                         |                         |                         |  |
| Anti-Rabbit IgG (H+L), made in goat                | <a href="#">CI-1000</a> | <a href="#">FI-1000</a> | <a href="#">DI-1488</a> | <a href="#">DI-1549</a> | <a href="#">DI-1594</a> | <a href="#">DI-1649</a> | <a href="#">CY-1300</a> | <a href="#">CY-1500</a> |  |
| Anti-Rat IgG (H+L), made in rabbit                 |                         | <a href="#">FI-4000</a> |                         |                         |                         |                         |                         |                         |  |
| Anti-Rat IgG (H+L), mouse adsorbed, made in rabbit |                         | <a href="#">FI-4001</a> |                         |                         |                         |                         |                         |                         |  |
| Anti-Rat IgG, made in goat                         |                         |                         |                         |                         |                         |                         | <a href="#">CY-4300</a> | <a href="#">CY-4500</a> |  |
| Anti-Goat IgG (H+L), made in horse                 |                         |                         | <a href="#">DI-3088</a> |                         | <a href="#">DI-3094</a> |                         |                         |                         |  |
| Anti-Goat IgG (H+L), made in rabbit                | <a href="#">CI-5000</a> | <a href="#">FI-5000</a> |                         |                         |                         |                         |                         |                         |  |
| VectaFluor™ Anti-Rabbit IgG, made in horse         |                         |                         | <a href="#">DI-1788</a> |                         | <a href="#">DI-1794</a> |                         |                         |                         |  |
| VectaFluor™ Anti-Mouse IgG, made in horse          |                         |                         | <a href="#">DI-2788</a> |                         | <a href="#">DI-2794</a> |                         |                         |                         |  |
| VectaFluor™ Anti-Goat IgG, made in horse           |                         |                         | <a href="#">DI-3788</a> |                         | <a href="#">DI-3794</a> |                         |                         |                         |  |

| Anti-Human Fluochrome-Labeled Secondary Antibodies   |                         |                         |              |              |              |              |     |     |  |
|--|-------------------------|-------------------------|--------------|--------------|--------------|--------------|-----|-----|--|
| Product  | AMCA                    | Fluorescein             | DyLight® 488 | DyLight® 549 | DyLight® 594 | DyLight® 649 | Cy3 | Cy5 |  |
| Anti-Human IgG (H+L), made in goat                   |                         | <a href="#">FI-3000</a> |              |              |              |              |     |     |  |
| Anti-Human IgE, epsilon chain specific, made in goat |                         | <a href="#">FI-3040</a> |              |              |              |              |     |     |  |
| Anti-Human IgG, gamma chain specific, made in goat   |                         | <a href="#">FI-3080</a> |              |              |              |              |     |     |  |
| Anti-Human IgM, mu chain specific, made in goat      |                         | <a href="#">FI-3020</a> |              |              |              |              |     |     |  |
| Anti-Human Kappa Chain, made in goat                 | <a href="#">CI-3060</a> | <a href="#">FI-3060</a> |              |              |              |              |     |     |  |
| Anti-Human Lambda Chain, made in goat                | <a href="#">CI-3070</a> | <a href="#">FI-3070</a> |              |              |              |              |     |     |  |

2

3

| Tertiary Reagents                               |                         |                         |                         |                         |                         |                         |                         |                         |                         |
|---|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Product   | Biotin                  | AMCA                    | Fluorescein             | DyLight® 488            | DyLight® 549            | DyLight® 594            | DyLight® 649            | Cy3                     | Cy5                     |
| VectaFluor™ Excel Amplified Anti-Rabbit IgG Kit |                         |                         |                         | <a href="#">DK-1488</a> |                         | <a href="#">DK-1594</a> |                         |                         |                         |
| VectaFluor™ Excel Amplified Anti-Mouse IgG Kit  |                         |                         |                         | <a href="#">DK-2488</a> |                         | <a href="#">DK-2594</a> |                         |                         |                         |
| Streptavidin                                    |                         | <a href="#">SA-5008</a> | <a href="#">SA-5001</a> | <a href="#">SA-5488</a> | <a href="#">SA-5549</a> | <a href="#">SA-5594</a> | <a href="#">SA-5649</a> | <a href="#">SA-1300</a> | <a href="#">SA-1500</a> |
| Avidin  |                         | <a href="#">A-2008</a>  | <a href="#">A-2001</a>  |                         |                         |                         |                         |                         |                         |
| Avidin DCS                                      |                         |                         | <a href="#">A-2011</a>  |                         |                         |                         |                         |                         |                         |
| Anti-Streptavidin                               | <a href="#">BA-0500</a> |                         |                         | <a href="#">SP-4488</a> | <a href="#">SP-4549</a> |                         |                         |                         |                         |
| Anti-Avidin                                     | <a href="#">BA-0300</a> |                         |                         |                         |                         |                         |                         |                         |                         |

2

| 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) | <a href="#">DK-8818</a>  |
| VectaFluor™ Duet IF Double Labeling Kit - DyLight® 594 Anti-Rabbit (red) - DyLight® 488 Anti-Mouse (green) | <a href="#">DK-8828</a>  |
| Mouse on Mouse (M.O.M.) Kits   |                          |
| M.O.M.™ Basic Kit  | <a href="#">BMK-2202</a> |
| M.O.M.™ Fluorescein Kit  | <a href="#">FMK-2201</a> |

4

| Counterstain/Mount  |                        |
|---|------------------------|
| Product   | Cat. No.               |
| Non-Hardening Formula                                       |                        |
| VECTASHIELD® Mounting Medium                                | <a href="#">H-1000</a> |
| VECTASHIELD® Mounting Medium with DAPI                      | <a href="#">H-1200</a> |
| VECTASHIELD® Mounting Medium with PI                        | <a href="#">H-1300</a> |
| Hardening Formula   |                        |
| VECTASHIELD® HardSet™ Mounting Medium                       | <a href="#">H-1400</a> |
| VECTASHIELD® HardSet™ Mounting Medium with DAPI             | <a href="#">H-1500</a> |
| VECTASHIELD® HardSet™ Mounting Medium with TRITC-Phalloidin | <a href="#">H-1600</a> |