Quality Matters: Bethyl DyLight Antibody Conjugates

Criteria for Fluorescent Probe Selection

The use of fluorescent probes has become a mainstream technique to detect specific molecular targets in both cell-free and cell-based assays. In addition, there is now a clear trend towards high throughput assay systems, where multiple signals are simultaneously detected from arrays of samples, and collected data are routinely normalized and compared. These types of assays require high quality reagents and optimized experimental conditions for high specificity and sensitivity. Therefore, the following important factors have to be considered in selecting fluorescent probes or dyes.

High Fluorescence Intensity – The brightness of the fluorescence emission of a particular fluorophore is proportional to the product of its extinction coefficient and its quantum yield. The brighter the dye, the higher is the sensitivity.

Wide Stokes Shift – This is the separation between the absorption and the emission maxima of the dye. Assuming everything else being equal, the wider separation results in higher signal-to-background ratio and increases the overall sensitivity of the detection.

Narrow Absorption and Emission Spectra – The absorption and emission spectra of a particular dye usually overlap, resulting in fluorescence quenching. Dyes with smaller spectral overlaps enable multi-color detection of different molecular targets in the same experiment.

Good Photostability – Photobleaching is a result of gradual breaking of covalent bonds in the fluorophore molecules upon exposure to intense light. This reduces signal intensity over time and can lead to irreproducible experimental data.

Excellent Aqueous solubility – Aggregation or precipitation of the antibody-dye conjugates has to be avoided in order to minimize non-specific binding which, in turn, can potentially result in fluorescence quenching.

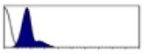
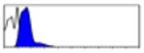
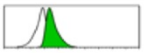
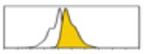
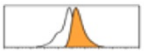
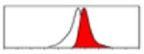
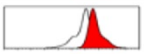
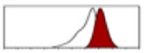
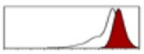
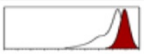
DyLight Dyes: The Superior Alternative to Other Dyes

The DyLight™ fluorophores are a series of fluorescent molecules designed to address the assay requirements described above. This series exhibits fluorescence emission covering essentially the entire visible light spectrum, and their spectral and molecular characteristics are shown in Table 1. These fluorophores exhibit higher, or at least comparable, fluorescence intensities and greater photostabilities compared to their analogous Alexa Fluor®, CyDye™ and Li-COR dyes.

Finally, antibodies labeled with DyLight Fluors have good aqueous solubility and stability over a broad pH (pH 4-9). DyLight conjugates are, therefore, ideal for simultaneous identification and quantitation of multiple molecular targets in cell-free and cell-based assays.

Table 1. Spectral Properties of DyLight™ Fluorescent Dyes.

Spectral Properties of DyLight Fluorescent Dyes. Each dye name and spectrum-image links to a dedicated information page that includes the detailed spectra, example data and literature references.

Emission	Fluor	Ex/Em†	See Spectra	ε††	Spectrally Similar Dyes
Blue	DyLight 350	353/432		15K	Alexa Fluor* 350, AMCA
Blue	DyLight 405	400/420		30K	Alexa Fluor 405, Cascade Blue*
Green	DyLight 488	493/518		70K	Alexa Fluor 488, fluorescein, FITC
Yellow	DyLight 550	562/576		150K	Alexa Fluor 546, Alexa Fluor 555, Cy3*,TRITC
Red	DyLight 594	593/618		80K	Alexa Fluor 594, Texas Red*
Red	DyLight 633	638/658		170K	Alexa Fluor 633
Red	DyLight 650	652/672		250K	Alexa Fluor 647, Cy5*
Near IR	DyLight 680	692/712		140K	Alexa Fluor 680, Cy5.5*
Near IR	DyLight 755	754/776		220K	Alexa Fluor 750
Infrared	DyLight 800	777/794		270K	IRDye* 800

†Excitation and emission maxima in nanometers (+/- 4nm) in phosphate-buffered saline (PBS)
††Molar extinction coefficient (M^{-1} cm^{-1}) at the absorption maximum

DyLight Spectral Chart used with permission from Thermo Fisher Scientific.

Applications

Bethyl scientists performed comparison experiments that demonstrate the superiority of Bethyl DyLight™ conjugated secondary antibodies over other antibody fluorphore conjugates from other manufacturers, such as Alexa Fluor® antibody conjugates. See Figures 1-8 below.

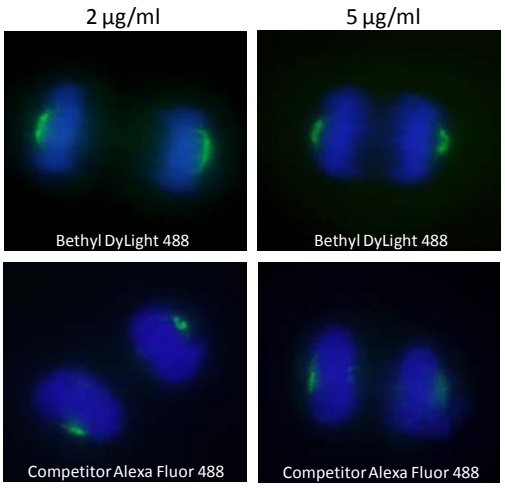


Figure 1. ICC images demonstrate Bethyl DyLight™ 488 conjugated antibodies (top row) are significantly brighter than corresponding cells stained with Alexa Fluor® 488 conjugated antibodies from a competing manufacturer (bottom row). NBF-fixed HeLa cells probed with Anti-ASPM (Bethyl cat. no. IHC-00058) and detected with Goat Anti-Rabbit IgG (h+l) conjugated to DyLight™ 488 (Bethyl cat. no. A120-601D2) (top row) and with a secondary conjugate Alexa Fluor 488® from a competing manufacturer (bottom row). All exposure conditions were identical.

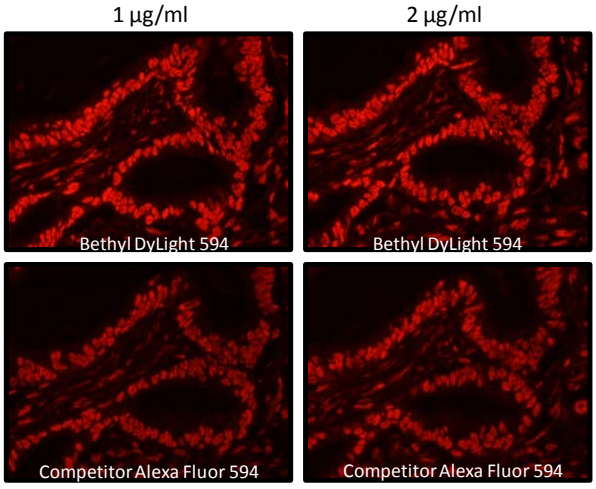


Figure 2. IHC images demonstrate Bethyl DyLight™ 594 conjugated antibodies (top row) are significantly brighter than corresponding cells stained with Alexa Fluor® 594 conjugated antibodies from a competing manufacturer (bottom row). FFPE colon carcinoma cells probed with Anti-Matrin3 (Bethyl cat. no. IHC-00081) and detected with Goat Anti-Rabbit IgG (h+l) conjugated to DyLight 594™ (Bethyl cat. no. A120-601D4) (top row) and with a secondary conjugate Alexa Fluor 594® from a competing manufacturer (bottom row). All exposure conditions were identical.

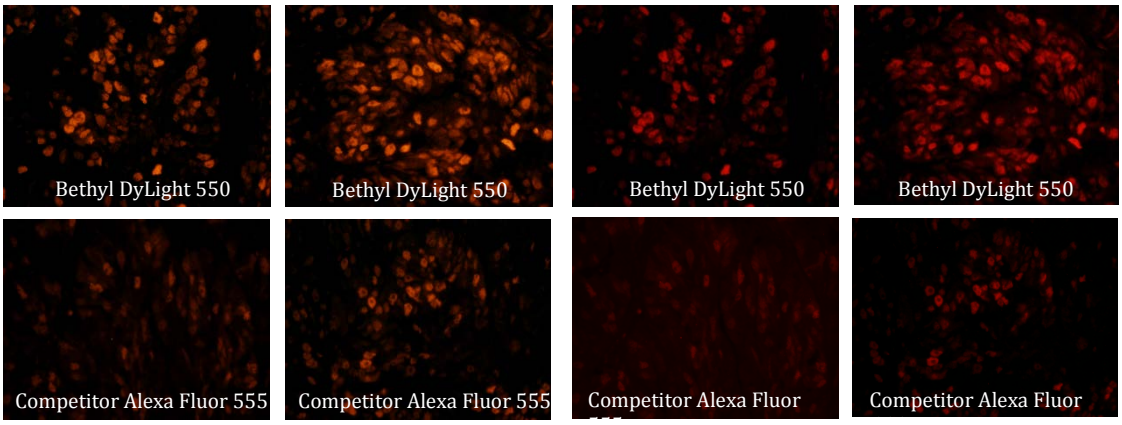


Figure 3. IHC images demonstrate that Bethyl DyLight™ 550 conjugated antibodies (top row) are significantly brighter than corresponding cells stained with Alexa Fluor® 555 conjugated antibodies from a competing manufacturer (bottom row). FFPE colon carcinoma tissue was probed with Rabbit Anti-PCNA antibody (Bethyl Cat. no. IHC-00012) and detected with 1 µg/ml and 5 µg/ml of Goat Anti-Rabbit IgG (h+l) conjugated to DyLight™ 550 (Bethyl Lab. Cat. No. A120-601D3) (top row) and with Goat Anti-Rabbit IgG (h+l) Alexa Fluor® 555 (bottom row). Images were taken using TRITC (Rhodamine) and Texas Red filters (left and right panels, respectively). All exposure conditions were identical.

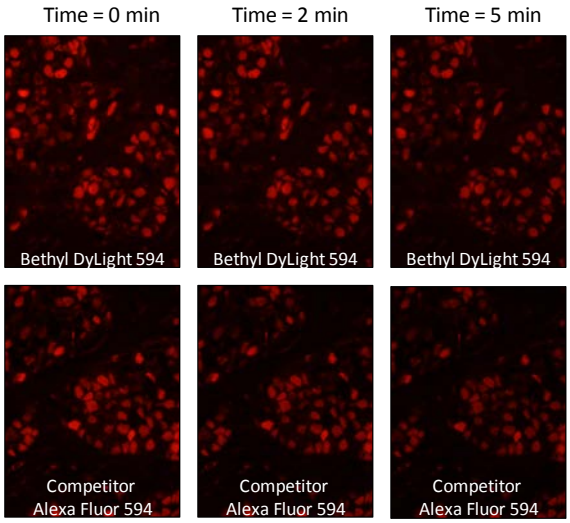


Figure 4. IHC images demonstrate that Bethyl DyLight™ 594 (top row) photostability is as good if not better when compared to Alexa Fluor® 594 conjugate (bottom row). FFPE human breast carcinoma tissue was probed with Rabbit Anti-PCNA antibody (Bethyl cat. no. IHC-00012) and detected with Goat Anti-Rabbit IgG (h+l) conjugated to DyLight™ 594 (Bethyl cat. no. A120-601D4) (top row) and Goat Anti-Rabbit IgG (h&+l) Alexa Fluor® 594 (bottom row). Stained sections were continuously exposed to light and images captured at the indicated time intervals. All exposure conditions were identical.

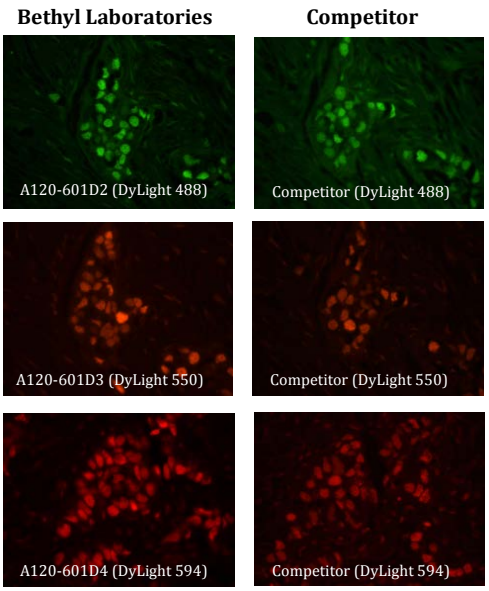


Figure 5. IHC images demonstrate Bethyl DyLight™ conjugated antibodies (left column) are significantly brighter than DyLight™ conjugated antibodies from a competitor (right column). FFPE human breast carcinoma tissue was probed with Rabbit Anti-PCNA (Bethyl cat. no. IHC-00012) and detected with Goat Anti Rabbit IgG (h+l) conjugated to DyLight™ 488 (top row), DyLight™ 550 (middle row), and DyLight™ 594 (bottom row). All exposure conditions were identical.

Figure 6. Isoelectric focusing images of Rabbit IgG detected with Goat anti-Rabbit IgG conjugated to DyLight™ 488 (blue), DyLight™ 550 (green), DyLight™ 650 (yellow), and DyLight™ 755 (red). The use of DyLight conjugates for detection of proteins in IEF can be used as a superior alternative to conventional stains for protein detection.

Dyes	Filter	Lighting
DyLight 488	F525	Blue LED
DyLight 550	UV06	Green LED
DyLight 650	705M	Red LED
DyLight 755	LY800	IR740

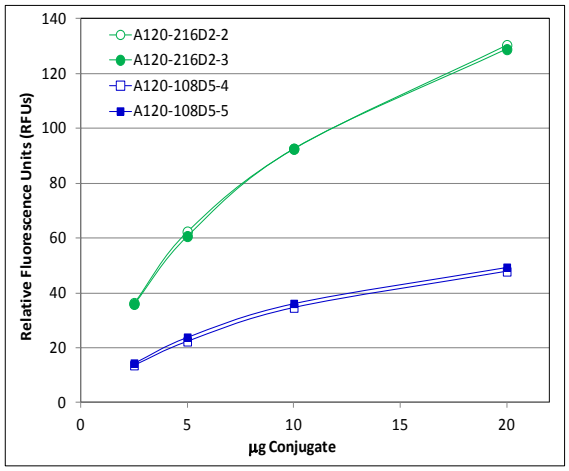


Figure 7. Excellent lot-to-lot performance of selected Dylight™ conjugates by direct ELISA. Wells were coated with rabbit IgG and detected with two different lots of Goat Anti-Rabbit IgG Dylight™ conjugates. The relative fluorescence units (RFUs) of the samples were read and converted into net RFUs by subtracting the RFUs of the isotype controls. A120-216D2 is a Fab'2 Donkey Anti-Rabbit IgG h+l cross adsorbed antibody conjugated to DyLight™ 488. A120-108D5 is a Donkey Anti-Rabbit IgG h+l IgG conjugated to DyLight™ 650.

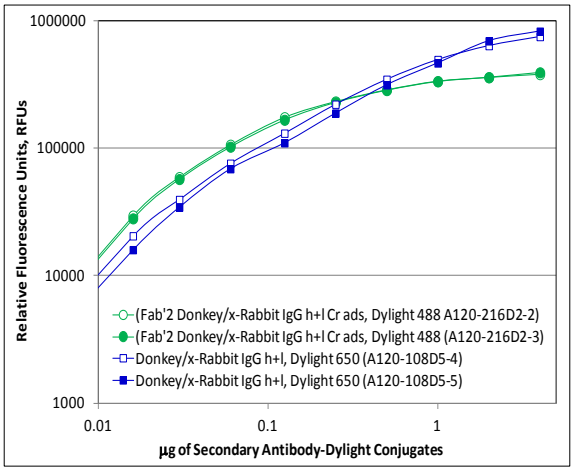


Figure 8. Excellent lot-to-lot performance of selected Dylight™ conjugates in flow cytometry assays. Para-formaldehyde fixed and permeabilized cells were stained with Rabbit Anti-Rictor antibody (A300-459A) followed by detection with two different lots of Anti-Rabbit IgG DyLight™ conjugates. The relative fluorescence units (RFUs) of the isotype controls were subtracted from the raw RFUs of the samples to obtain the net RFUs.

Bethyl Quality Assurance

Since 1972, Bethyl Laboratories has focused efforts to manufacture high quality antibodies. Every Bethyl product ships with a 100% guarantee. Coupled with the superior spectral characteristics of DyLight™ fluorophores, Bethyl offers an array of high quality antibody-DyLight™ conjugates spanning essentially the entire visible spectrum. Your experiment is important to you; choose Bethyl antibodies with confidence.

Product List

To view a list of Bethyl's DyLight™ fluorophores conjugated secondary antibodies, please click here. You can filter your results using the check boxes. Bethyl has nearly 500 antibodies conjugated to the following DyLight Fluors: DyLight 350, DyLight 488, DyLight 550, DyLight 594, DyLight 650, DyLight 680, DyLight 755, DyLight 800.

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