Atto Labels and Their Conjugates – Versatile, Bright and Stable Tools for Imaging

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Atto dyes are a comprehensive series of fluorescent dyes, covering the entire spectrum of visible light and matching the most common output wavelengths of excitation light sources, especially mercury and xenon lamps, but also common lasers (Figure 1). Atto dyes provide the brightest fluorescence with narrow fluorescence emission spectra. These properties enable the parallel imaging of different targets in cells, tissues or other biological samples.

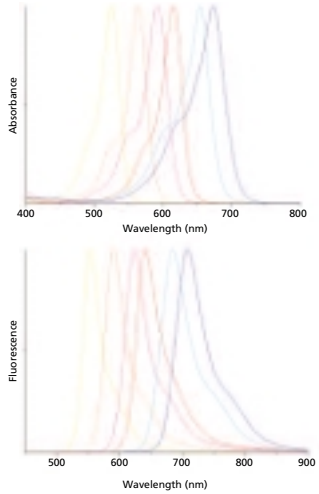

Figure 1. Absorption and emission spectra of Atto 520, Atto 565, Atto 590, Atto 610, Atto 655 and Atto 680.

Atto 488, a superior alternative to the widely used fluorescein, provides tremendously improved photostability and more intense fluorescence. It can be excited with the same light sources as fluorescein dyes or Alexa 488, and the same optical filter sets and instrument settings are used to record the emission. Proteins can be labeled with numerous Atto 488 labels without significant quenching. Atto 550 and Atto 565 are efficiently excited by HeNe lasers (543 nm) and are used as alternatives to rhodamine dye, Cy™3, or Alexa 550, offering higher intensity and very good photostability. Atto 635, 647, or 655 are well suited for excitation with HeNe or Krypton lasers, similar to Cy™5 or Alexa 555.

Autofluorescence of biological samples or support media can be a serious limitation for sensitivity and specificity of fluorescent techniques like immunohistochemistry and ELISA assays. Since most of the fluorescence of biological samples comes from shorter wavelengths, the impact of autofluorescence decreases with longer excitation and detection wavelengths. With excitation maxima up to 740 nm and emission maxima up to 764 nm, Atto dyes provide a set of tools to circumvent problems with autofluorescence.

Antibody and other fluorescent conjugates

To provide high quality conjugates with ideal intensity and low background, we optimized the labeling of antibodies with our innovative series of Atto labels. Secondary antibodies, the general workhorses for immunochemistry, are offered as conjugates with several of Atto labels as well as Mega labels, which are characterized by a large gap between excitation and emission maxima (Tables 1 and 2). For most of the Atto labels, streptavidin and biotin conjugates are also available.

Table 1. Mega labels and their spectroscopic properties.

Name	λ_{max} abs [nm]	λ_{max} em [nm]	ε_{max} [l/mol cm]
Fluorescent Red Mega 480	480	640	40,000
Fluorescent Red Mega 485	485	559	20,000
Fluorescent Red Mega 500	500	612	90,000
Fluorescent Red Mega 520	520	664	50,000

Table 2. Antibody conjugates available from stock.

Label	Goat anti-mouse IgG	Goat anti-rabbit IgG	Goat anti-human IgG	Rabbit anti-chicken IgG
Atto 488	18772	62197	52526	
Atto 550	43394	44328		
Atto 590		68919		50913
Atto 647	50185	40839		
Mega 485	12708	38376		
Mega 520	39304	02295		

Multiple staining with single excitation light

Parallel staining of different structures or target molecules in biological tissues or other samples can be complicated by overlaps of fluorescence signals. Thus labels with clearly distinct emission spectra are preferred. For this purpose the wide selection of labels offers a variety of suitable combinations (Figures 1-4).

Mega labels, if combined with Atto dyes, enable the visualization of different structures with just one excitation source. Figure 2 shows an application of Atto 488 and a Mega 485-labeled antibody in confocal microscopy. Such combinations can also be used on conventional fluorescence microscopes, using mercury lamp, even using standard filter sets optimized for conventional fluorophores (Figure 3).

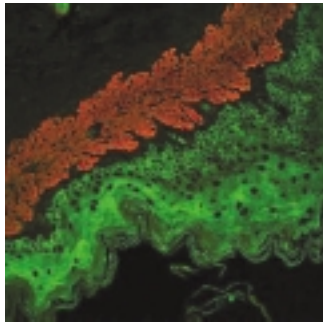

Figure 2. *Rat stomach: Actin stained with mouse anti-smooth muscle α-actin antibody and Atto 488 anti-mouse IgG (green), cytokeratin stained with polyclonal rabbit anti-cytokeratin and Mega 485 anti-rabbit IgG (red), both labels are excited by just one laser (Argon laser).*

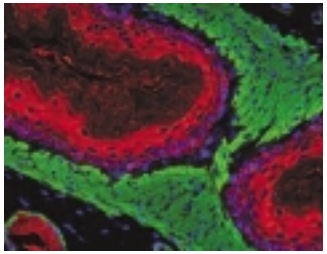

Figure 3. *Rat stomach: Actin stained with mouse anti-smooth muscle α-actin antibody and Atto 488 anti-mouse IgG (green), cytokeratin stained with polyclonal rabbit anti-cytokeratin and Mega 520 anti-rabbit IgG (red), counterstained with DAPI (blue). Courtesy of Jacob Zbaeren, Inselspital Bern, Switzerland.*

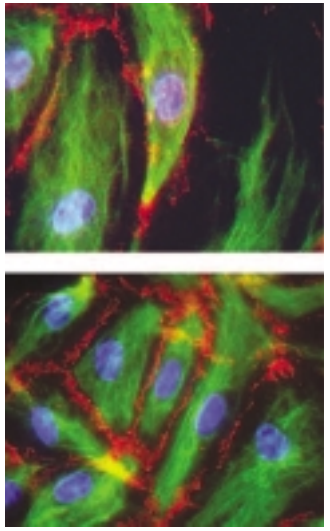

Figure 4. *Human endothelial cells: Vimentin stained with mouse anti-vimentin and Atto 550 anti-mouse IgG (green), cadherine stained with rabbit anti-cadherine antibody and Atto 655 goat anti-rabbit IgG (red). Counterstained with DAPI (blue).*

Stability

For well-known and commonly used labels like fluorescein, photostability is limited. Photostability becomes even more important with the increasing use of laser excitation, confocal and two-photon illumination, the increasing sensitivity of methods down to the single molecule level, and the tracking of processes over time in living cells. Atto labels and conjugates have more rigid structures, which make them exceptionally photostable, thereby, outperforming some of the most widely used dyes.

Ordering Information

Product	Description	Unit
41051-1MG-F	Atto 488	1 mg
42424-1MG-F	Atto 550	1 mg
75784-1MG-F	Atto 565	1 mg
08968-1MG-F	Atto 635	1 mg
93711-1MG-F	Atto 655	1 mg

For additional information on individual products, visit our homepage at ***sigma-aldrich.com***