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empowering the spirit of science

Conjugated Antibody Guide

Applications of ATTO dye fluorophores:

Conjugated antibody guide for multiplex immunostaining

By Alomone Labs

Alomone Labs

At Alomone Labs we have a diverse product portfolio that we have optimized specifically for use in immuno-colocalization and multiplex immunofluorescence. These reagents include directly conjugated and unconjugated antibodies, and labeled toxins. Our products are developed entirely in-house and undergo rigorous QC with lot-specific testing. We have a specialism in research tools for membrane proteins and cover neuroscience, cancer, cardiovascular, immunology, stem cells, metabolism, development and cancer research fields.

Introduction to fluorochrome labeled antibodies

A fluorochrome absorbs light energy of a specific wavelength, which is referred to as its excitation spectrum. It subsequently re-emits light energy at a longer wavelength that is known as its emission spectrum. Differences in spectra account for the disparate colors observed in experimental applications.

Fluorochrome or fluorescent dye labeled antibodies are excellent tools for helping to detect and visualize target antigens in tissues and cells for immunofluorescent (IF) techniques, flow cytometry (FACS) and multiplex applications. Primary antibodies can either be directly conjugated to a fluorescent dye, and bind to the antigen, or used in an unconjugated format whereby a labeled secondary is used for detection in an additional step. The latter can amplify the fluorescent signal, as more than one secondary antibody typically binds to the unlabeled primary.

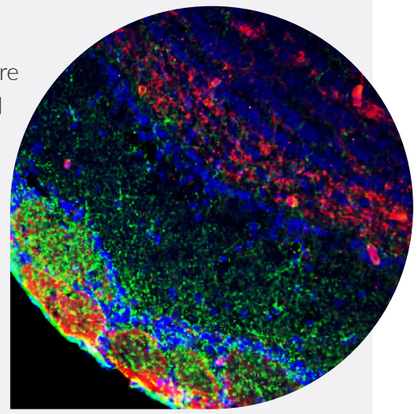
Advantages of using fluorochrome conjugated primaries

The use of a directly conjugated primary antibody has the advantage of streamlining your experiment as it eliminates the need for a secondary antibody. In addition, it enables the use of multiple primary antibodies from the same species for multicolor detection, reducing the non-specific staining that would be observed with secondary antibody cross-reactivity (e.g. mouse-on-mouse staining).

Combining fluorophores with differing emission spectra in this way is highly applicable for use in immuno-colocalization, sometimes known as multiplex immunofluorescence (IF) or immunostaining, which we focus on in this guide. Here, the co-occurrence of two or more proteins is determined in the same sub-cellular structure or tissue, with sequential antibody application procedures.

ATTO dyes**

ATTO dyes, are a new generation of proprietary fluorophore that are characterized by strong absorption (high extinction coefficient) and a high fluorescence quantum yield. Many common fluorescent labels (FITC, Alexa-Fluor dyes) can deteriorate in the presence of light radiation used in microscopy and similar techniques. This can also occur in the dark if they are exposed to relatively small concentrations of ozone present in a laboratory atmosphere. In contrast, ATTO dyes show very high photo-stability in these conditions, compared to their predecessors.



ATTO-conjugated primary antibodies are particularly suitable for multiplex applications, multi-colour detection IF, and FACS. In addition, ATTO-590 and above can be excited with wavelengths > 600 nm that result in significant decreases in background autofluorescence, improving imaging sensitivity.

ATTO versus Alexa Fluor dyes

ATTO dyes are equivalent to their Alexa Fluor dye counterparts, but typically offer brighter fluorescent signals and a number of other advantages:

- **Higher photo-stability**
- **Longer signal lifetimes**
- **Brighter fluorescent signals**

At Alomone labs we have ~100 ATTO-conjugated primary antibodies, and several ATTO-conjugated toxins. What's more, we offer custom conjugation to our customers.

ATTO dye fluorochrome reference table

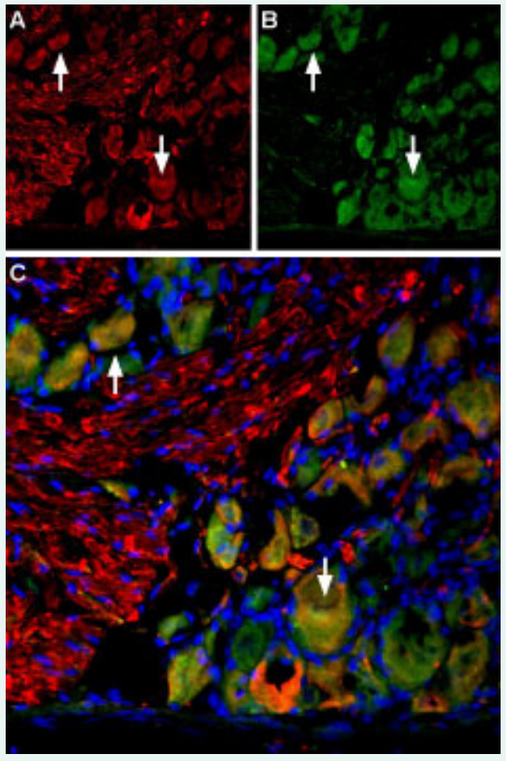
Fluorochrome	Excitation Range	Max Emission (λfl)	Relative brightness	Equivalent dyes (unsuitable for combination in multi-color experiments)	Notes
ATTO-488 (Fluorescent GREEN)	480 - 515 nm	520 nm	Bright	FITC, GFP, AF-488, FAM	Very hydrophilic
ATTO-550 (Orange)	540 - 565 nm	576 nm	Bright	Rhodamine 6G, Cy3, TAMRA, AF-550	Moderately Hydrophillic
ATTO-590 (Red)	575 - 610 nm	622 nm	Bright	Texas Red, AF-594	Moderately Hydrophillic
ATTO-594 (Red)	580 - 615 nm	626 nm	Bright	Texas Red, AF-594	Very hydrophilic
ATTO-633 (Red)	610 - 645 nm	651 nm	Bright	AF-633	Moderately Hydrophillic

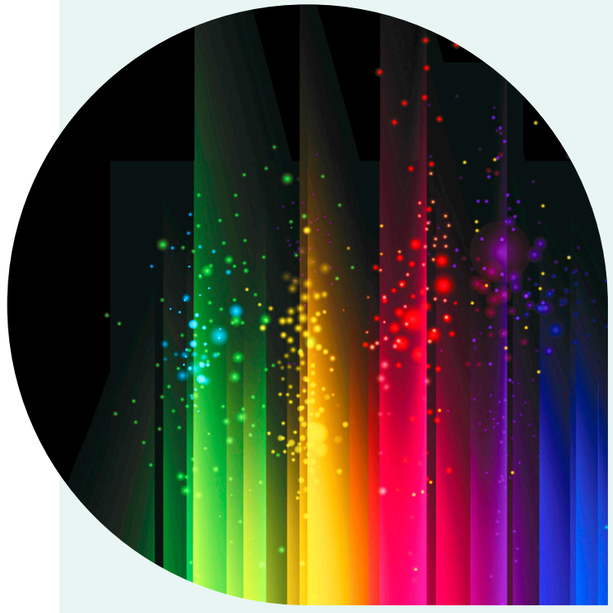
Product Highlight: ATTO-conjugated primaries

Anti-VGLUT2-ATTO Fluor-594 Antibody (#AGC-036-AR) + Anti-NK1R (extracellular)-ATTO Fluor-488 Antibody (#ATR-001-AG)

Multiplex immunostaining of VGLUT2 and NK1R in rat dorsal root ganglion (DRG)

Immunohistochemical staining of perfusion-fixed frozen rat dorsal root ganglion (DRG) sections using Anti-VGLUT2-ATTO Fluor-594 Antibody (#AGC-036-AR), (1:60) and Anti-Neurokinin 1 Receptor (NK1R) (extracellular)-ATTO Fluor-488 Antibody (#ATR-001-AG), (1:60). A. VGLUT2 staining (red). B. NK1 receptor staining (green). C. Merge of the two images demonstrates co-localization in some neuronal bodies (arrows point at examples). Cell nuclei are stained with DAPI (blue).





Reducing non-specific staining in multiplex immunofluorescence staining

A common problem faced in multiplex immunostaining techniques is non-specific staining. This can be a consequence of the cross-over between the species of the test tissue and antibody, or alternatively the hosts in which the multiple primary antibodies applied were raised.

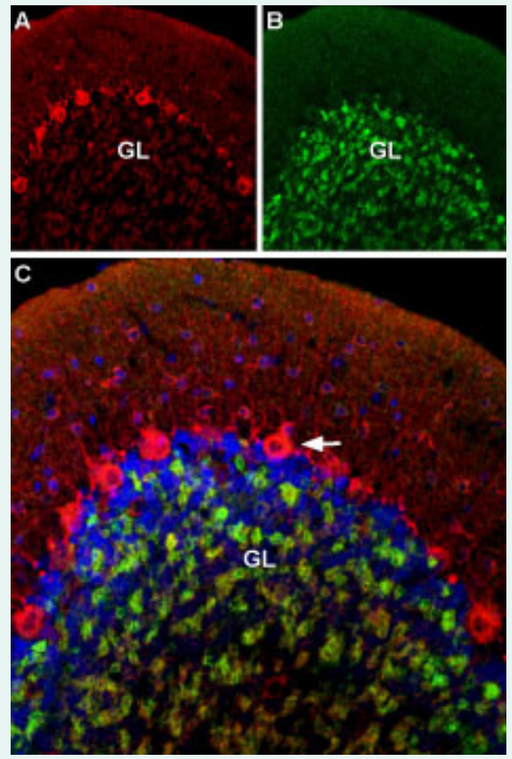
Alomone labs offers guinea pig-raised antibodies providing an additional option and more flexibility for our users. These antibodies can be used in combination with antibodies commonly raised in species like mouse, rabbit, and goat to address this specific issue. Furthermore, they can be combined with any of our ATTO-conjugated antibodies in complex immunostaining protocols.

Product Highlight: Guinea Pig-Raised Antibodies

Guinea Pig Anti-Cav1.2 (CACNA1C) Antibody (#AGP-001) + Anti-GABA(A) α1 Receptor (extracellular)-ATTO Fluor-488 Antibody (#AGA-001-AG)

Multuplex immunostaining of Cav1.2 and GABA(A) α1 Receptor in rat cerebellum

Immunohistochemical staining of rat cerebellum using Guinea pig Anti-Cav1.2 (CACNA1C) Antibody (#AGP-001) and Anti-GABA(A) α1 Receptor (extracellular)-ATTO Fluor-488 Antibody (#AGA-001-AG). A. Cav1.2 (red) is detected mostly in Purkinje cells (arrow). B. In the same section, GABA(A) α1 Receptor (green) is observed in the granule layer. C. Merge of the two images suggests some colocalization between Cav1.2 and GABA(A) α1 Receptor in the rat granule layer, but only Cav1.2 appears in Purkinje cells.



** ATTO dyes are used under license from from ATTO-TEC GmBH https://www.atto-tec.com

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