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Product data sheet

Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 A-11001

Catalog Number

Details		Species Reactivity	
Size	500 μL	Species reactivity	Mouse
Host/Isotope	Goat / IgG	Tested Applications	Dilution *
Class	Polyclonal	Flow Cytometry (Flow)	1-10 µg/mL
Туре	Secondary Antibody	Immunocytochemistry (ICC)	1 μg/mL
Immunogen	Gamma Immunoglobins Heavy and Light chains	Immunofluorescence (IF)	1 μg/mL
Target Class	lgG	Published Applications	See 23 publications below
Cross Adsorption	Against human IgG and human serum prior to conjugation	Immunocytochemistry (ICC) Immunohistochemistry (Frozen) (IHC (F))	See 7 publications below
Antibody Form	Whole Antibody	Immunohistochemistry (IHC)	See 10 publications below
Conjugate	Alexa Fluor® 488	Western Blot (WB)	See 4 publications below
Form	Liquid	Immunohistochemistry (Paraffin)	See 6 publications below
Concentration	2 mg/ml	(IHC (P))	See o publications below
Purification	purified	Immunohistochemistry - Free Floating (IHC (Free))	See 2 publications below
Storage buffer	PBS, pH 7.5	Flow Cytometry (Flow)	See 4 publications below
Contains	5mM sodium azide	Miscellaneous PubMed (MISC)	See 612 publications below
Storage Conditions	4° C, store in dark	* Suggested working dilutions are given as a guide only. It is recommusing appropriate negative and positive controls.	nended that the user titrate the product for use in their own experiment

Product specific information

To minimize cross-reactivity, these goat anti-mouse IgG whole antibodies have been cross-adsorbed against human IgG and human serum. Crossadsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins. For a highly cross-adsorbed secondary antibody equivalent, please see product Cat. No. A11029. Alexa Fluor dyes are among the most trusted fluorescent dyes available today. InvitrogenTM Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 488 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot. Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg /mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Background/Target Information

We offer an extensive line of Invitrogen[™] secondary antibody conjugates with well-characterized specificity and labeled with a wide selection of premium fluorescent dyes, including Invitrogen™ Alexa Fluor™ fluorescent dyes. Fluorescent secondary antibody conjugates are useful in the detection, sorting, or purification of its specified target and ideal for fluorescence microscopy and confocal laser scanning microscopy, flow cytometry, and fluorescent western

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detection. The breadth of fluorescent markers we offer allows our reagents to be tailored to almost any fluorescent detection system. Secondary antibodies may be provided in three formats: whole IgG, divalent F(ab')2 fragments, and monovalent Fab fragments. Because of the high degree of conservation in the structure of many immunoglobulin domains, most class-specific secondary antibodies must be affinity-purified and cross-adsorbed to achieve minimal crossreaction with other immunoglobulins. Our secondary antibody conjugates are most commonly prepared by immunizing the host animal with a pooled population of immunoglobulins from the target species and can be further purified and modified (e.g., immunoaffinity chromatography, antibody fragmentation, label conjugation, etc.) to generate highly specific reagents. In the first round of purification, whole immunoglobulins binding to the immunizing antibody are recovered and mainly consist of the ~150-kDa IgG class. Further purification, for example, with Protein A or G, removes all unwanted immunoglobulin classes except the affinity-purified antibodies that react with the target-specific immunoglobulin heavy and/or light chains.

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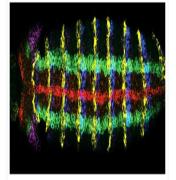
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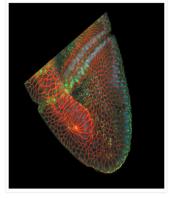
Product Images For Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488

Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

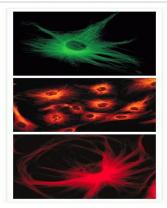


Simultaneous detection of expression of five genes in a whole-mount Drosophila embryo by fluorescence in situ hybridization (FISH) with five RNA probes. Red: sog labeled using aminoallyl UTP (Product # A21663, A32765) and Alexa Fluor® 647 succinimidyl ester (Product # A-20006, A20106). Green: ind labeled with DNP, followed by rabbit antidinitrophenyl-KLH IgG antibody (Product # A-6430) prelabeled with the Zenon® Alexa Fluor® 555 Rabbit IgG Labeling Kit (Product # Z-25305). Blue: en labeled with biotin and detected with HRP-streptavidin and Alexa Fluor® 405 tyramide (TSA[™] Kit 39, Product # T30952). Yellow: wg labeled with digoxigenin and detected with sheep anti-digoxigenin IgG antibody and Alexa Fluor® 594 Donkey Anti-Sheep IgG antibody (Product # A-11016). Magenta: msh labeled with fluorescein and detected with mouse anti-fluorescein/Oregon Green® IgG2a antibody (Product # A-6421) and Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001, A11029). Image contributed by Dave Kosman and Ethan Bier, University of California, San Diego.

Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

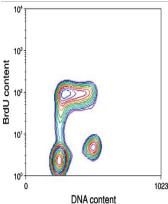


Formation of the cephalic furrow in the anterior end of a developing Drosophila melanogaster embryo visualized with the help of several fluorescent stains. A primary antibody to neurotactin was visualized using a red-fluorescent Cy3 dye secondary antibody (Amersham Pharmacia Biotech Ltd.). Primary antibodies to plasma membrane-bound myosin and to nuclear-localized even-skipped (Eve) protein were visualized with green-fluorescent Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001) and Alexa Fluor® 488 Goat Anti-Rabbit IgG antibody (Product # A-11008), respectively. The nuclei were stained with blue-fluorescent Hoechst 33342 (Product # H1399, H3570, H21492). The sample was prepared by Eric Wieschaus, and the imaging was performed by Joe Goodhouse of Princeton University.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Microtubules of bovine pulmonary artery endothelial cells tagged with mouse monoclonal anti-a-tubulin antibody (Product # A11126) and subsequently probed with: Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001, top panel), Alexa Fluor® 546 Goat Anti-Mouse IgG antibody (Product # A-11005, bottom panel). These images were acquired using a fluorescein bandpass optical filter set, a rhodamine bandpass optical filter set, and a Texas Red bandpass optical filter set, respectively.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in Flow

Cells were treated with 10 µM 5-bromo-2 and acute-deoxyuridine (BrdU, Product # B23151) in culture medium for one hour, then pelleted and fixed with cold 70% ethanol. After treatment with RNase and 4 M HCI (to denature the DNA), the cells were labeled with anti-BrdU (Product # A21300) and detected using green-fluorescent Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001). In addition, the cells were labeled with red-fluorescent propidium iodide (Product # P1304MP, P3566, P21493) to assess the total cellular DNA content. The cells were analyzed by flow cytometry using 488 nm excitation; the fluorescent signals were collected at ~525 nm for the Alexa Fluor® 488 dye and at ~675 nm for propidium iodide. Increased BrdU incorporation is indicative of actively proliferating cells.

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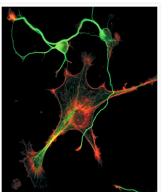
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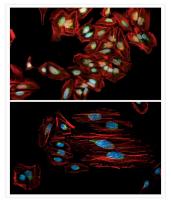
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Filamentous structures of neuronal cells in a rat cerebellum were fluorescently labeled to differentiate the cell types. The cerebellum section was probed with primary antibodies to neurofilament and glial fibrillary acidic proteins (GFAP) and subsequently visualized with the green-fluorescent Alexa Fluor® 488 Goat Anti-Mouse IgG (Product # A-11001) and redorange-fluorescent Alexa Fluor® 568 Goat Anti-Rabbit IgG (Product # A-11011) antibodies. This confocal micrograph was contributed by Gillian Davidson, Andrew Hubbard and Chris Guerin, Neurotoxicology Group, M.R.C Toxicology Unit, University of Leicester, Leicester, U.K.



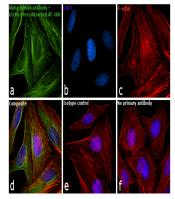
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Confocal micrograph of the cytoskeleton of a mixed population of granule neurons and glial cells. The F-actin was stained with red-fluorescent Texas Red®-X phalloidin (Product # T7471). The microtubules were detected with a mouse monoclonal anti-ß-tubulin primary antibody and subsequently visualized with the green-fluorescent Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001). The image was contributed by Jonathan Zmuda, Immunomatrix, Inc.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Golgi and actin staining in HeLa cells. Golgi in fixed and permeabilized HeLa cells labeled with anti-golgin-97 monoclonal antibody (Product # A-21270) and visualized with green-fluorescent Alexa Fluor® 488 Goat-Anti-Mouse IgG (Product # A-11001). Actin was stained with red-fluorescent Alexa Fluor® 594 phalloidin (Product # A12381); nuclei were stained with blue-fluorescent DAPI (Product # D1306, D3571, D21490). Treatment with Image-iT® FX signal enhancer (Product # I36933) largely eliminates nonspecific dye binding (bottom) as compared to untreated slide (top).



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal Antibody (Product # A11126). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/ml Mouse primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 conjugate (Product # A-11001) was used at a concentration of 1ug/ml in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: green). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Rhodamine Phalloidin (Product # R415, 1:300) (Panel c: red). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.

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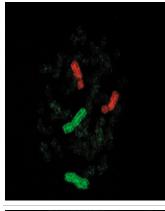
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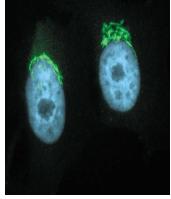
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Increased label specificity and resolution afforded by Image-iT® FX signal enhancer. Fixed and permeabilized MRC-5 human lung fibroblast cells were labeled with mouse anti-human Golgin-97 primary antibody (Product # A-21270), then visualized with either fluorescein goat anti-mouse IgG (F2761, top row) or Alexa Fluor® 488 Goat Anti-Mouse IgG (Product # A-11001, bottom row). Cells on the right were treated with Image-iT® FX signal enhancer (Product # I36933) prior to antibody incubation. (Product # A-21270).



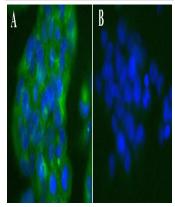
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

Labeled paint probes hybridized to human metaphase chromosomes. A biotinylated chromosome 5 probe was detected with Alexa Fluor® 594 streptavidin (Product # S-11227), and a digoxigenin-labeled chromosome 2 probe detected with mouse anti-digoxigenin in combination with Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001). Image contributed by Joop Wiegant, Leiden University Medical Center, Leiden, The Netherlands.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

HeLa cells labeled with anti-golgin-97 antibody and detected using Alexa Fluor® 488 Goat Anti-Mouse IgG antibody. Fixed, permeabilized HeLa cells were labeled with anti-golgin-97 monoclonal antibody (Product # A-21270) and detected using Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001). The cells were counterstained with DAPI (D1306, D3571, D21490). (Product # A-21270).



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in IF

NF-kappa-B (p65) Clone: 572 Product # 436700. Immunoflorescence of 4% PFA-fixed Hela cells (A) NF-kappa-B (P65) Clone: 572 Product # 436700 shown in green, Alexa Fluor® 488 secondary antibody. Nuclei are shown in blue with DAPI. (B) No primary antibody (negative control), Alexa Fluor® 488 secondary antibody, and Nuclei are shown in blue with DAPI.

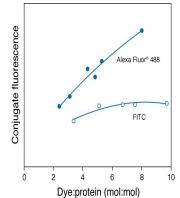
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Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in N/A

Conjugate fluorescence is determined by measuring the fluorescence quantum yield of the conjugated dye relative to that of a reference dye and multiplying by the dye: protein labeling ratio.

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23 Immunocytochemistry F	References
Species / Dilution	Summary
Not Applicable / 2 mg/ml	A11001 was used in immunocytochemistry to develop a protocol to generate expandable and multipotent induced cardiac progenitor cells from mouse adult fibroblasts
	Nature protocols (May 2017; 12: 1029) "Generation of multipotent induced cardiac progenitor cells from mouse fibroblasts and potency testing in ex vivo mouse embryos." Author(s):Lalit PA,Rodriguez AM,Downs KM,Kamp TJ PubMed Article URL:http://dx.doi.org/10.1038/nprot.2017.021
	A-11001 was used in immunocytochemistry to characterize the transition from free-swimming to adherent Trichomonas vaginalis
Not Applicable / Not Cited	Cellular microbiology (Oct 2013; 15: 1707) "The actin-based machinery of Trichomonas vaginalis mediates flagellate-amoeboid transition and migration across host tissue." Author(s):Kusdian G,Woehle C,Martin WF,Gould SB PubMed Article URL:http://dx.doi.org/10.1111/cmi.12144
	A-11001 was used in immunocytochemistry to investigate how early embryonic-like cells are promoted by downregulating replication-dependent chromatin assembly
Not Applicable / Not Cited	Nature structural and molecular biology (Sep 2015; 22: 662) "Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly." Author(s):Ishiuchi T,Enriquez-Gasca R,Mizutani E,Boškovi A,Ziegler-Birling C,Rodriguez-Terrones D,Wakayama T, Vaquerizas JM,Torres-Padilla ME PubMed Article URL:http://dx.doi.org/10.1038/nsmb.3066
	A11001 was used in immunocytochemistry to identify microRNAs regulating gene expression associated with muscle development
Not Applicable / Not Cited	International journal of biological sciences (Nov 2017; 13: 157) "Regulatory Axis of miR-195/497 and HMGA1-Id3 Governs Muscle Cell Proliferation and Differentiation." Author(s):Qiu H,Zhong J,Luo L,Tang Z,Liu N,Kang K,Li L,Gou D PubMed Article URL:http://dx.doi.org/10.7150/ijbs.17440
	A-11001 was used in immunocytochemistry to elucidate the physiological significance of S857 phosphorylation.
Not Applicable / Not Cited	Biochemistry (Aug 2012; 51: 6786) "Activity-dependent phosphorylation of dynamin 1 at serine 857." Author(s):Xie W,Adayev T,Zhu H,Wegiel J,Wieraszko A,Hwang YW PubMed Article URL:http://dx.doi.org/10.1021/bi2017798
	A11001 was used in immunocytochemistry to generate an induced pluripotent stem cell line from an essential thrombocythemia patient with a heterozygous MPL V501L mutation
Not Applicable / 1:500	Stem cell research (Jan 2017; 18: 57) "Generation of human iPSCs from an essential thrombocythemia patient carrying a V501L mutation in the MPL gene." Author(s):Liu S,Ye Z,Gao Y,He C,Williams DW,Moliterno A,Spivak J,Huang H,Cheng L PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2016.12.012
	A-11001 was used in immunocytochemistry to identify an interaction between TOR kinase and inositol polyphosphate signaling systems that regulates storage lipid accumulation
Not Applicable / 1:1000	The Plant cell (Sep 2016; null: null) "Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control and lipid metabolism in Chlamydomonas." Author(s):Couso I,Evans B,Li J,Liu Y,Ma F,Diamond S,Allen DK,Umen JG PubMed Article URL:http://dx.doi.org/10.1105/tpc.16.00351

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2 Immunohistochemistry -	Free Floating References
Species / Dilution	Summary

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