





Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647

Catalog Number A-21244 Product data sheet

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Details		S
Size	500 μL	S
Host/Isotope	Goat / IgG	Ī
Class	Polyclonal	F
Туре	Secondary Antibody	li
Immunogen	Gamma Immunoglobins Heavy and Light chains	li li
Target Class	IgG	
Cross Adsorption	Against human IgG, human serum, mouse IgG, mouse serum and bovine serum	li F
Antibody Form	Whole Antibody	li
Conjugate	Alexa Fluor® 647	lı (l
Form	liquid	V
Concentration	2 mg/ml	N
Purification	purified	F
Storage buffer	PBS, pH 7.5	* 5
Contains	5mM sodium azide	us
Storage Conditions	4° C, store in dark	

Species Reactivity		
Species reactivity	Rabbit	
Tested Applications	Dilution *	
Flow Cytometry (Flow)	1-10 μg/mL	
Immunocytochemistry (ICC)	4 μg/mL	
Immunofluorescence (IF)	4 μg/mL	
Published Applications		
rubiisiieu Applications		
Immunohistochemistry (IHC)	See 6 publications below	
Immunohistochemistry - Free Floating (IHC (Free))	See 2 publications below	
Immunocytochemistry (ICC)	See 7 publications below	
Immunohistochemistry (Frozen) (IHC (F))	See 1 publications below	
Western Blot (WB)	See 2 publications below	
Miscellaneous PubMed (MISC)	See 39 publications below	
Flow Cytometry (Flow)	See 2 publications below	

^{*} Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

To minimize cross-reactivity, these goat anti-rabbit IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. Cross-adsorption 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. Alexa Fluor dves are among the most trusted fluorescent dves available today. Invitrogen™ Alexa Fluor 647 dve is a nearinfrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 dye is pH-insensitive over a wide molar rangé. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 647 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 cross-reaction 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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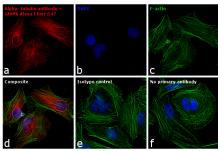
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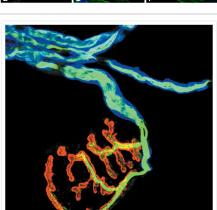
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Product Images For Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647





Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21244) in IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 647 conjugate was performed using HeLa cells stained with alpha Tubulin Rabbit Polyclonal Antibody (Product # PA5-16891). 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 primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 647 conjugate (Product # A-21244) was used at a concentration of 4 µg/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: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). 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.

Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21244) in IF

Acetylcholine receptors were stained with the tetramethylrhodamine conjugate of a-bungarotoxin (Product # T-1175). Axons were labeled with a primary antibody against neurofilaments and visualized with a green-fluorescent, fluorescein-labeled secondary antibody. Myelin was labeled using an antibody against P0 protein, visualized with Alexa Fluor® 647 goat anti-mouse IgG antibody (Product # A-21244) and pseudocolored blue. Image contributed by Felipe Court and R.R. Ribchester, University of Edinburgh, Scotland.

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6 Immunohistochemistry F	References
Species / Dilution	Summary
Not Applicable / 1:500	A21244 was used in immunohistochemistry to quantify soluble triggering receptor expressed on myeloid cells 2 and amyloid levels in Alzheimer's disease mouse model brains
	Frontiers in aging neuroscience (Sep 2017; 9: null) "Increase of TREM2 during Aging of an Alzheimer's Disease Mouse Model Is Paralleled by Microglial Activation and Amyloidosis." Author(s):Brendel M,Kleinberger G,Probst F,Jaworska A,Overhoff F,Blume T,Albert NL,Carlsen J,Lindner S,Gildehaus FJ, Ozmen L,Suárez-Calvet M,Bartenstein P,Baumann K,Ewers M,Herms J,Haass C,Rominger A PubMed Article URL:http://dx.doi.org/10.3389/fnagi.2017.00008
	A-21244 was used in immunohistochemistry to study the interactions of beta-amyloid deposits and microglia in a mouse model of Alzheimer's disease
Not Applicable / 1:500	PloS one (Mar 2016; 10: null) "Fibrillar amyloid plaque formation precedes microglial activation." Author(s):Jung CK,Keppler K,Steinbach S,Blazquez-Llorca L,Herms J PubMed Article URL:http://dx.doi.org/10.1371/journal.pone.0119768
	A-21244 was used in immunohistochemistry to analyze impairment of the regeneration of neuromuscular junctions by an inducible depletion of adult skeletal muscle stem cells
Not Applicable / 1:1500	eLife (Aug 2015; 4: null) "Inducible depletion of adult skeletal muscle stem cells impairs the regeneration of neuromuscular junctions." Author(s):Liu W,Wei-LaPierre L,Klose A,Dirksen RT,Chakkalakal JV PubMed Article URL:http://dx.doi.org/10.7554/eLife.09221
	A-21244 was used in immunohistochemistry to develop and characterize 'spaghetti monster' fluorescent proteins
Not Applicable / 1:500	Nature methods (Jun 2015; 12: 568) "High-performance probes for light and electron microscopy." Author(s):Viswanathan S,Williams ME,Bloss EB,Stasevich TJ,Speer CM,Nern A,Pfeiffer BD,Hooks BM,Li WP,English BP, Tian T,Henry GL,Macklin JJ,Patel R,Gerfen CR,Zhuang X,Wang Y,Rubin GM,Looger LL PubMed Article URL:http://dx.doi.org/10.1038/nmeth.3365
	A-21244 was used in immunohistochemistry to report that vein-derived endothelial tip cells contribute to emerging arteries in zebrafish
Not Applicable / 1:500	Nature communications (Dec 2014; 5: null) "Arteries are formed by vein-derived endothelial tip cells." Author(s):Xu C,Hasan SS,Schmidt I,Rocha SF,Pitulescu ME,Bussmann J,Meyen D,Raz E,Adams RH,Siekmann AF PubMed Article URL:http://dx.doi.org/10.1038/ncomms6758
	A-21244 was used in immunohistochemistry to examine LYVE-1 expression in corneal lymphatics.
Not Applicable / 20 µg/ml	FASEB journal: official publication of the Federation of American Societies for Experimental Biology (Feb 2012; 26: 808) "Discontinuous LYVE-1 expression in corneal limbal lymphatics: dual function as microvalves and immunological hot spots." Author(s):Nakao S,Zandi S,Faez S,Kohno R,Hafezi-Moghadam A PubMed Article URL:http://dx.doi.org/10.1096/fj.11-183897
2 Immunohistochemistry -	Free Floating References
Species / Dilution	Summary
Not Applicable / 1:500	A21244 was used in immunohistochemistry - free floating to report that aggregation-induced increases in phosphatase-activating domain exposure and oligomerization are common features among all tau isoforms
	Neurobiology of aging (Nov 2016; 47: 113) "Analysis of isoform-specific tau aggregates suggests a common toxic mechanism involving similar pathological conformations and axonal transport inhibition." Author(s):Cox K,Combs B,Abdelmesih B,Morfini G,Brady ST,Kanaan NM PubMed Article URL:http://dx.doi.org/10.1016/j.neurobiolaging.2016.07.015

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A21244 was used in immunohistochemistry - free floating to test if anti-Nogo-A treatment increases hippocampal neurogenesis after stroke

Frontiers in neuroscience (Aug 2017; 10: null)

Not Applicable / 1:1000
Frontiers in neuroscience (Aug 2017; 10: null)
"Anti-Nogo-A Immunotherapy Does Not Alter Hippocampal Neurogenesis after Stroke in Adult Rats."
Author(s):Shepherd DJ,Tsai SY,O'Brien TE,Farrer RG,Kartje GL
PubMed Article URL:http://dx.doi.org/10.3389/fnins.2016.00467

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Species / Dilution	Summary
Not Applicable / 1:1000	A21244 was used in immunocytochemistry to optimize the generation of murine cardiomyocytes from pluripotent stem cells
	Stem cell reviews (Dec 2016; 12: 731) "Simple Monolayer Differentiation of Murine Cardiomyocytes via Nutrient Deprivation-Mediated Activation of - Catenin." Author(s):Hofbauer P,Jung JP,McArdle TJ,Ogle BM PubMed Article URL:http://dx.doi.org/10.1007/s12015-016-9678-0
Not Applicable / 1:1000	A-21244 was used in immunocytochemistry to describe a role for APRIN in homologous recombination
	Nucleic acids research (Dec 2016; 44: 10879) "Roles for APRIN (PDS5B) in homologous recombination and in ovarian cancer prediction." Author(s):Couturier AM,Fleury H,Patenaude AM,Bentley VL,Rodrigue A,Coulombe Y,Niraj J,Pauty J,Berman JN,Dellaire G,Di Noia JM,Mes-Masson AM,Masson JY PubMed Article URL:http://dx.doi.org/10.1093/nar/gkw921
	A21244 was used in immunocytochemistry to analyze the proteomic profile of synaptic clefts
Not Applicable / Not Cited	Cell (Aug 2016; 166: 1295) "Proteomic Analysis of Unbounded Cellular Compartments: Synaptic Clefts." Author(s):Loh KH,Stawski PS,Draycott AS,Udeshi ND,Lehrman EK,Wilton DK,Svinkina T,Deerinck TJ,Ellisman MH,Stevens B Carr SA,Ting AY PubMed Article URL:http://dx.doi.org/10.1016/j.cell.2016.07.041
	A21244 was used in immunocytochemistry to generate cell line to examine the molecular events controlling HIV expression in the microglia
Not Applicable / Not Cited	Journal of neurovirology (Feb 2017; 23: 47) "Immortalization of primary microglia: a new platform to study HIV regulation in the central nervous system." Author(s):Garcia-Mesa Y,Jay TR,Checkley MA,Luttge B,Dobrowolski C,Valadkhan S,Landreth GE,Karn J,Alvarez-Carbonell D PubMed Article URL:http://dx.doi.org/10.1007/s13365-016-0499-3
	A-21244 was used in immunocytochemistry to propose using three-dimensional collagen scaffolds to study anti-glioma therapeutics
Not Applicable / Not Cited	Oncotarget (Aug 2016; 7: 56904) "A three-dimensional collagen scaffold cell culture system for screening anti-glioma therapeutics." Author(s):Lv D,Yu SC,Ping YF,Wu H,Zhao X,Zhang H,Cui Y,Chen B,Zhang X,Dai J,Bian XW,Yao XH PubMed Article URL:http://dx.doi.org/10.18632/oncotarget.10885
Not Applicable / Not Cited	A21244 was used in immunocytochemistry to find that Legionella pneumophila has evolved to modulate mechanistic target of rapamycin-dependent lipogenesis in order to replicate
	PLoS pathogens (Dec 2016; 12: null) "MTOR-Driven Metabolic Reprogramming Regulates Legionella pneumophila Intracellular Niche Homeostasis." Author(s):Abshire CF,Dragoi AM,Roy CR,Ivanov SS PubMed Article URL:http://dx.doi.org/10.1371/journal.ppat.1006088
Not Applicable / Not Cited	A21244 was used in immunocytochemistry to show that LAMP-2 is essential for STX17 expression and for fusion of autophagasomes with lysosomes
	Biology open (Oct 2016; 5: 1516) "LAMP-2 is required for incorporating syntaxin-17 into autophagosomes and for their fusion with lysosomes." Author(s):Hubert V,Peschel A,Langer B,Gröger M,Rees A,Kain R PubMed Article URL:http://dx.doi.org/10.1242/bio.018648

1 Immunohistochemistry (Frozen) References

Species / Dilution Summary

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	A21244 was used in immunohistochemistry - frozen section to investigate how swelling and the extracelluar matrix affect intratumoral stresses
Not Applicable / 1:800	Neoplasia (New York, N.Y.) (Dec 2016; 18: 732) "Hyaluronan-Derived Swelling of Solid Tumors, the Contribution of Collagen and Cancer Cells, and Implications for Cancer Therapy." Author(s):Voutouri C,Polydorou C,Papageorgis P,Gkretsi V,Stylianopoulos T PubMed Article URL:http://dx.doi.org/10.1016/j.neo.2016.10.001
2 Western Blot References	
Species / Dilution	Summary
Not Applicable / 1:200	A21244 was used in western blot to test if mitochondrial calcium uniporter concentrated at the mitochondria-sarcoplasmic reticulum interface promotes calcium transfer
	The Journal of biological chemistry (Oct 2016; 291: 23343) "Strategic Positioning and Biased Activity of the Mitochondrial Calcium Uniporter in Cardiac Muscle." Author(s):De La Fuente S,Fernandez-Sanz C,Vail C,Agra EJ,Holmstrom K,Sun J,Mishra J,Williams D,Finkel T,Murphy E, Joseph SK,Sheu SS,Csordás G PubMed Article URL:http://dx.doi.org/10.1074/jbc.M116.755496
	A-21244 was used in western blot to test if targeting tropomysin receptor kinase B reduces radiation-induced cognitive deficit
Not Applicable / 1:1000	Experimental neurology (May 2016; 279: 178) "Cognitive impairments following cranial irradiation can be mitigated by treatment with a tropomyosin receptor kinase B agonist." Author(s):Yang P,Leu D,Ye K,Srinivasan C,Fike JR,Huang TT PubMed Article URL:http://dx.doi.org/10.1016/j.expneurol.2016.02.021
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Species / Dilution	Summary
Not Applicable / Not Cited	Journal of the American Chemical Society (Apr 2009; 131: 4967) "Robust fluorescent detection of protein fatty-acylation with chemical reporters." Author(s):Charron G,Zhang MM,Yount JS,Wilson J,Raghavan AS,Shamir E,Hang HC PubMed Article URL:http://dx.doi.org/10.1021/ja810122f
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Not Applicable / Not Cited	BioTechniques (Feb 2006; 40: 220) "Direct production and purification of T7 phage display cloned proteins selected and analyzed on microarrays." Author(s):Nowak JE,Chatterjee M,Mohapatra S,Dryden SC,Tainsky MA PubMed Article URL:http://dx.doi.org/null
Not Applicable / Not Cited	Stem cells (Dayton, Ohio) (Jul 2007; 25: 1779) "Isolation, characterization, and differentiation to hepatocyte-like cells of nonparenchymal epithelial cells from adult human liver." Author(s):Duret C,Gerbal-Chaloin S,Ramos J,Fabre JM,Jacquet E,Navarro F,Blanc P,Sa-Cunha A,Maurel P,Daujat-Chavanieu M PubMed Article URL:http://dx.doi.org/10.1634/stemcells.2006-0664
Not Applicable / Not Cited	Proceedings of the National Academy of Sciences of the United States of America (Dec 2011; 108: 21081) "Bleaching/blinking assisted localization microscopy for superresolution imaging using standard fluorescent molecules." Author(a): Burnotto DT Songupta P Dai V Lippingott Schwartz J Kachar R

fusion peptides."

Author(s):Barry C,Key T,Haddad R,Duncan R

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"Features of a spatially constrained cystine loop in the p10 FAST protein ectodomain define a new class of viral

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