

## Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546

Catalog Number **A-11010**

Product data sheet

Details		Species Reactivity	
Size	500 µL	Species reactivity	Rabbit
Host/Isotope	Goat / IgG	<b>Tested Applications</b>	
Class	Polyclonal	Flow Cytometry (Flow)	1-10 µg/mL
Type	Secondary Antibody	Immunocytochemistry (ICC)	4 µg/mL
Immunogen	Gamma Immunoglobins Heavy and Light chains	Immunofluorescence (IF)	4 µg/mL
Target Class	IgG	<b>Published Applications</b>	
Cross Adsorption	Against human IgG, human serum, mouse IgG, mouse serum and bovine serum	Immunohistochemistry (Frozen) (IHC (F))	See 4 publications below
Antibody Form	Whole Antibody	Immunocytochemistry (ICC)	See 2 publications below
Conjugate	Alexa Fluor® 546	Western Blot (WB)	See 2 publications below
Form	liquid	Immunohistochemistry (IHC)	See 3 publications below
Concentration	2 mg/ml	Immunohistochemistry (Paraffin) (IHC (P))	See 1 publications below
Purification	purified	Miscellaneous PubMed (MISC)	See 71 publications below
Storage buffer	PBS, pH 7.5	* 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.	
Contains	5mM sodium azide		
Storage Conditions	4° C, store in dark		

### 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 may be the presence of endogenous immunoglobulins. Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 546 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 546 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 546 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 546 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')<sub>2</sub> 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.

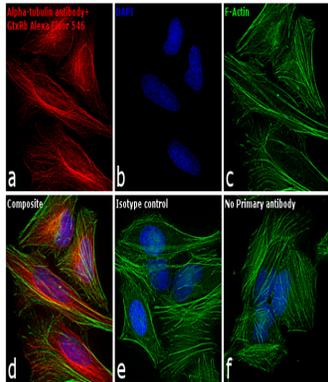
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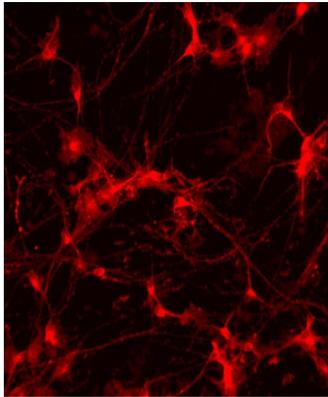
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## Product Images For Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546



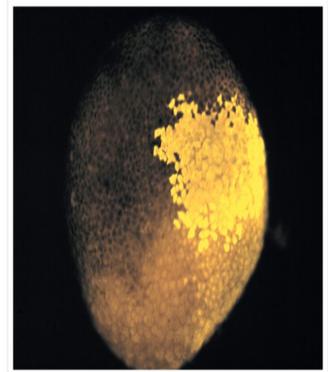
### Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11010) in IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-11010) 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 of mouse primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-11010) was used at concentration of 4µg/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-11010) in IF

Immunofluorescence analysis of PSD95 (red) in human motor neurons derived from iPSCs. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.2% Triton X-100 in PBS for 10 minutes, and blocked with 5% donkey serum in PBS for 15 minutes at room temperature. Cells were stained with a PSD95 rabbit monoclonal antibody (Product # 700902) diluted at 1:1000 in 5% donkey serum overnight at 4°C, and then incubated with a Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 546 conjugate (Product # A-11010) at a dilution of 1:1000 for 1 hour at room temperature (green). Note: Data courtesy of Innovators Program.



### Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11010) in IF

Embryos at the 8-cell stage were injected with anionic, lysine-fixable Cascade Blue® 10,000 MW dextran in one dorsal-animal blastomere and allowed to develop to various stages before being fixed. The Cascade Blue® dye, which serves as an antigen in this technique, was detected with an antibody to the Cascade Blue® dye and subsequently visualized with a secondary antibody conjugated to the Alexa Fluor® 546 dye (Product # A-11010). This photographic image was taken using a bandpass filter set appropriate for rhodamine. The image was contributed by Paul Wilson, Cornell University Medical College, New York, and Greg Cox, Molecular Probes, Inc.

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## PubMed References For Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546

### 4 Immunohistochemistry (Frozen) References

Species / Dilution	Summary
	A11010 was used in immunohistochemistry - frozen section to generate AAV vectors designed for specific expression in cells of the CNS using minimal promoters to drive gene expression
Not Applicable / 1:200	Frontiers in neuroanatomy (Aug 2017; 11: null) <b>"Adeno-Associated Viral Vectors Serotype 8 for Cell-Specific Delivery of Therapeutic Genes in the Central Nervous System."</b> Author(s):Pignataro D,Sucunza D,Vanrell L,Lopez-Franco E,Dopeso-Reyes IG,Vales A,Hommel M,Rico AJ,Lanciego JL,Gonzalez-Aseguinolaza G PubMed Article URL: <a href="http://dx.doi.org/10.3389/fnana.2017.00002">http://dx.doi.org/10.3389/fnana.2017.00002</a>
Not Applicable / 1:1000	A11010 was used in immunohistochemistry - frozen section to show decreased Bmal1 in GLAST-positive astrocytes alters circadian locomotor behavior and cognition in mice Nature communications (Feb 2017; 8: null) <b>"Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling."</b> Author(s):Barca-Mayo O,Pons-Espinal M,Follert P,Armirotti A,Berdondini L,De Pietri Tonelli D PubMed Article URL: <a href="http://dx.doi.org/10.1038/ncomms14336">http://dx.doi.org/10.1038/ncomms14336</a>
Not Applicable / 1:2000	A-11010 was used in immunohistochemistry - frozen section to study the benefits of heterochronic parabiosis in young and old mice Nature communications (Nov 2016; 7: null) <b>"A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood."</b> Author(s):Rebo J,Mehdipour M,Gathwala R,Causey K,Liu Y,Conboy MJ,Conboy IM PubMed Article URL: <a href="http://dx.doi.org/10.1038/ncomms13363">http://dx.doi.org/10.1038/ncomms13363</a>
Not Applicable / Not Cited	A11010 was used in immunohistochemistry - frozen section to quantify biologically valuable micronutrients incorporated and distributed into the exogenously developing brain using cerebral organoids derived from human pluripotent stem cells PeerJ (Aug 2017; 5: null) <b>"Trace elements during primordial plexiform network formation in human cerebral organoids."</b> Author(s):Sartore RC,Cardoso SC,Lages YV,Paraguassu JM,Stelling MP,Madeiro da Costa RF,Guimaraes MZ,Pérez CA,Rehen SK PubMed Article URL: <a href="http://dx.doi.org/10.7717/peerj.2927">http://dx.doi.org/10.7717/peerj.2927</a>

### 2 Immunocytochemistry References

Species / Dilution	Summary
Not Applicable / 1:200	A11010 was used in immunocytochemistry to use nanosensor labeling to study embryonic stem cells-derived embryoid body in vivo Acta biomaterialia (Feb 2017; 49: 358) <b>"Real-time and non-invasive monitoring of embryonic stem cell survival during the development of embryoid bodies with smart nanosensor."</b> Author(s):Fu J,Wiraja C,Chong R,Xu C,Wang DA PubMed Article URL: <a href="http://dx.doi.org/10.1016/j.actbio.2016.11.027">http://dx.doi.org/10.1016/j.actbio.2016.11.027</a>
Not Applicable / 1:500	A11010 was used in immunocytochemistry to investigate 20nm citrate-coated and polyvinylpyrrolidone-coated silver nanoparticle-induced neurotoxicity Neurotoxicology (Dec 2016; 57: 45) <b>"Silver nanoparticles exhibit coating and dose-dependent neurotoxicity in glutamatergic neurons derived from human embryonic stem cells."</b> Author(s):Begum AN,Aguilar JS,Elias L,Hong Y PubMed Article URL: <a href="http://dx.doi.org/10.1016/j.neuro.2016.08.015">http://dx.doi.org/10.1016/j.neuro.2016.08.015</a>

### 2 Western Blot References

Species / Dilution	Summary
Not Applicable / Not Cited	A11010 was used in western blot to demonstrate the existence and functions of quaternary structures of SMS1 and SMS2 The Journal of biological chemistry (Jan 2017; 292: 1122) <b>"Carboxyl-terminal Tail-mediated Homodimerizations of Sphingomyelin Synthases Are Responsible for Efficient Export from the Endoplasmic Reticulum."</b> Author(s):Hayashi Y,Nemoto-Sasaki Y,Matsumoto N,Tanikawa T,Oka S,Tanaka Y,Arai S,Wada I,Sugiura T,Yamashita A PubMed Article URL: <a href="http://dx.doi.org/10.1074/jbc.M116.746602">http://dx.doi.org/10.1074/jbc.M116.746602</a>

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A11010 was used in western blot to develop methods to study vesicle-associated proteins and exocytosis in stellate astrocytes

Not Applicable / 1:2000

The Journal of general physiology (Jan 2017; 149: 149)

**"A novel method for culturing stellate astrocytes reveals spatially distinct Ca<sup>2+</sup> signaling and vesicle recycling in astrocytic processes."**

Author(s):Wolfes AC,Ahmed S,Awasthi A,Stahlberg MA,Rajput A,Magruder DS,Bonn S,Dean C  
PubMed Article URL:<http://dx.doi.org/10.1085/jgp.201611607>

### 3 Immunohistochemistry References

Species / Dilution	Summary
Not Applicable / 1:400	A11010 was used in immunohistochemistry to find that mice with a dysfunctional fibronectin-synergy motif suffer from surprisingly mild platelet adhesion and bleeding defects due to delayed thrombus formation after vessel injury  eLife (Jan 2017; 6: null) <b>"The fibronectin synergy site re-enforces cell adhesion and mediates a crosstalk between integrin classes."</b> Author(s):Benito-Jardón M,Klapproth S,Gimeno-LLuch I,Petzold T,Bharadwaj M,Müller DJ,Zuchtriegel G,Reichel CA,Costell M PubMed Article URL: <a href="http://dx.doi.org/10.7554/eLife.22264">http://dx.doi.org/10.7554/eLife.22264</a>
Not Applicable / 1:200	A-11010 was used in immunohistochemistry to test if IL-1beta and TNF-alpha are synthesized by overlapping or segregated populations of cells after ischemic stroke in mice  Journal of neuroinflammation (Oct 2008; 5: null) <b>"Interleukin-1beta and tumor necrosis factor-alpha are expressed by different subsets of microglia and macrophages after ischemic stroke in mice."</b> Author(s):Clausen BH,Lambertsen KL,Babcock AA,Holm TH,Dagnaes-Hansen F,Finsen B PubMed Article URL: <a href="http://dx.doi.org/10.1186/1742-2094-5-46">http://dx.doi.org/10.1186/1742-2094-5-46</a>
Not Applicable / 1:50	A11010 was used in immunohistochemistry to find a developmental role for Gsc in the delamination of otic neuroblasts  Proceedings of the National Academy of Sciences of the United States of America (Nov 2016; 113: E6840) <b>"Spemann organizer gene Goosecoid promotes delamination of neuroblasts from the otic vesicle."</b> Author(s):Kantarci H,Gerberding A,Riley BB PubMed Article URL: <a href="http://dx.doi.org/10.1073/pnas.1609146113">http://dx.doi.org/10.1073/pnas.1609146113</a>

### 1 Immunohistochemistry (Paraffin) References

Species / Dilution	Summary
Not Applicable / Not Cited	A11010 was used in immunohistochemistry - paraffin section to investigate the role of PD-L1 and T-cell infiltration in metastatic high-grade osteosarcoma  Cancer immunology, immunotherapy : CII (Jan 2017; 66: 119) <b>"Increased PD-L1 and T-cell infiltration in the presence of HLA class I expression in metastatic high-grade osteosarcoma: a rationale for T-cell-based immunotherapy."</b> Author(s):Sundara YT,Kostine M,Cleven AH,Bovée JV,Schilham MW,Cleton-Jansen AM PubMed Article URL: <a href="http://dx.doi.org/10.1007/s00262-016-1925-3">http://dx.doi.org/10.1007/s00262-016-1925-3</a>

### 71 Miscellaneous PubMed References

Species / Dilution	Summary
Not Applicable / Not Cited	Investigative ophthalmology and visual science (Mar 2012; 53: 1566) <b>"Multipotent stem cells from trabecular meshwork become phagocytic TM cells."</b> Author(s):Du Y,Roh DS,Mann MM,Funderburgh ML,Funderburgh JL,Schuman JS PubMed Article URL: <a href="http://dx.doi.org/10.1167/iovs.11-9134">http://dx.doi.org/10.1167/iovs.11-9134</a>
Not Applicable / Not Cited	The Journal of biological chemistry (Feb 2010; 285: 5931) <b>"Amino acid residues critical for endoplasmic reticulum export and trafficking of platelet-activating factor receptor."</b> Author(s):Hirota N,Yasuda D,Hashidate T,Yamamoto T,Yamaguchi S,Nagamune T,Nagase T,Shimizu T,Nakamura M PubMed Article URL: <a href="http://dx.doi.org/10.1074/jbc.M109.066282">http://dx.doi.org/10.1074/jbc.M109.066282</a>
Not Applicable / Not Cited	The Journal of biological chemistry (Aug 2005; 280: 30416) <b>"Connexin 43 interacts with zona occludens-1 and -2 proteins in a cell cycle stage-specific manner."</b> Author(s):Singh D,Solan JL,Taffet SM,Javier R,Lampe PD PubMed Article URL: <a href="http://dx.doi.org/10.1074/jbc.M506799200">http://dx.doi.org/10.1074/jbc.M506799200</a>
Not Applicable / Not Cited	The Journal of cell biology (Nov 2001; 155: 845) <b>"Role of heparan sulfate as a tissue-specific regulator of FGF-4 and FGF receptor recognition."</b> Author(s):Allen BL,Filla MS,Rapraeger AC PubMed Article URL: <a href="http://dx.doi.org/10.1083/jcb.200106075">http://dx.doi.org/10.1083/jcb.200106075</a>

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Not Applicable / Not Cited	The Journal of cell biology (Oct 2001; 155: 355) <b>"Distinct retrieval and retention mechanisms are required for the quality control of endoplasmic reticulum protein folding."</b> Author(s):Vashist S, Kim W, Belden WJ, Spear ED, Barlowe C, Ng DT PubMed Article URL: <a href="http://dx.doi.org/10.1083/jcb.200106123">http://dx.doi.org/10.1083/jcb.200106123</a>
Not Applicable / Not Cited	Molecular and cellular proteomics : MCP (Jun 2006; 5: 1131) <b>"Systematic characterization of nuclear proteome during apoptosis: a quantitative proteomic study by differential extraction and stable isotope labeling."</b> Author(s):Hwang SI, Lundgren DH, Mayya V, Rezaul K, Cowan AE, Eng JK, Han DK PubMed Article URL: <a href="http://dx.doi.org/10.1074/mcp.M500162-MCP200">http://dx.doi.org/10.1074/mcp.M500162-MCP200</a>
Not Applicable / Not Cited	The Journal of biological chemistry (Mar 2000; 275: 9461) <b>"The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex."</b> Author(s):Grimes JA, Nielsen SJ, Battaglioli E, Miska EA, Speh JC, Berry DL, Atouf F, Holdener BC, Mandel G, Kouzarides T PubMed Article URL: <a href="http://dx.doi.org/">http://dx.doi.org/</a>
Not Applicable / Not Cited	The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society (Jul 2009; 57: 687) <b>"Insoluble, speckled cytosolic distribution of retinoic acid receptor alpha protein as a marker of hepatic stellate cell activation in vitro."</b> Author(s):Mezaki Y, Yamaguchi N, Yoshikawa K, Miura M, Imai K, Itoh H, Senoo H PubMed Article URL: <a href="http://dx.doi.org/10.1369/jhc.2009.953208">http://dx.doi.org/10.1369/jhc.2009.953208</a>
Not Applicable / Not Cited	The Journal of biological chemistry (Aug 2001; 276: 33147) <b>"Synergistic activation of the Wnt signaling pathway by Dvl and casein kinase Iepsilon."</b> Author(s):Kishida M, Michiue T, Yamamoto H, Kishida S, Fukui A, Asashima M, Kikuchi A PubMed Article URL: <a href="http://dx.doi.org/10.1074/jbc.M103555200">http://dx.doi.org/10.1074/jbc.M103555200</a>
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