

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

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotope	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor® 488
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage Conditions	4° C, store in dark
RRID	AB_143165

Applications	Tested	Dilution	Published
Immunohistochemistry (IHC)	-	1:1000	20 Publications
Immunohistochemistry (Frozen) (IHC (F))	-		20 Publications
Immunocytochemistry (ICC)	✓	4 µg/mL	26 Publications
Immunohistochemistry (Paraffin) (IHC (P))	-	1:2000	8 Publications
Flow Cytometry (Flow)	✓	1-10 µg/mL	2 Publications
Western Blot (WB)	-		3 Publications
Immunohistochemistry - Free Floating (IHC (Free))	-	1:1000	1 Publication
ChIP assay (ChIP)	-		1 Publication
Miscellaneous PubMed (MISC)	-		472 Publications
Immunofluorescence (IF)	✓	4 µg/mL	

Product Specific Information

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ 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.

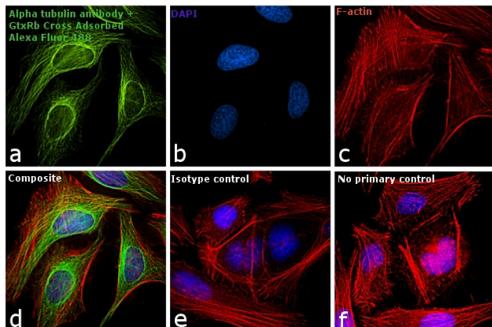
The goat anti-rabbit IgG whole antibody conjugates are most commonly prepared by immunizing the host animal with a pooled population of immunoglobulins from the target species and can be further purified and modified (e.g., immunoaffinity chromatography, antibody fragmentation, label conjugation, etc.) 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 with Protein A or G removes all immunoglobulin classes except IgG such that the affinity-purified antibodies react with IgG heavy chains and all classes of immunoglobulin light chains from rabbit. To minimize cross-reactivity, these goat anti-rabbit whole antibodies have been 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 where there is potential cross-reactivity with other primary antibodies or in immunohistochemistry experiments where there may be the presence of endogenous immunoglobulins. For a highly cross-adsorbed secondary antibody equivalent (or equivalent secondary antibody preparation), please see product catalog number: A11034.

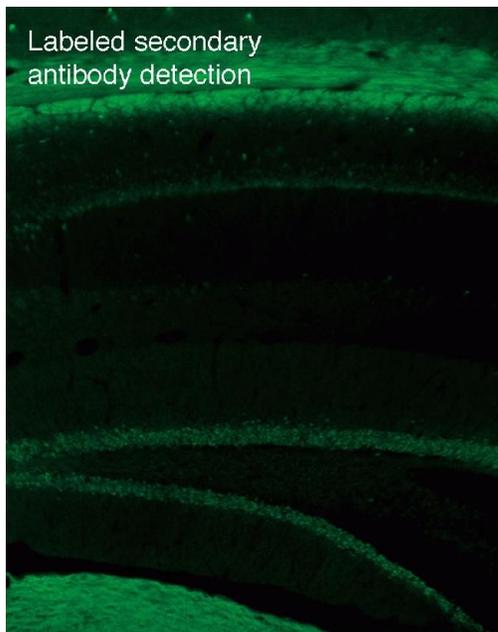
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 $\mu\text{g}/\text{mL}$ should be satisfactory for most immunohistochemistry and flow cytometry applications.

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

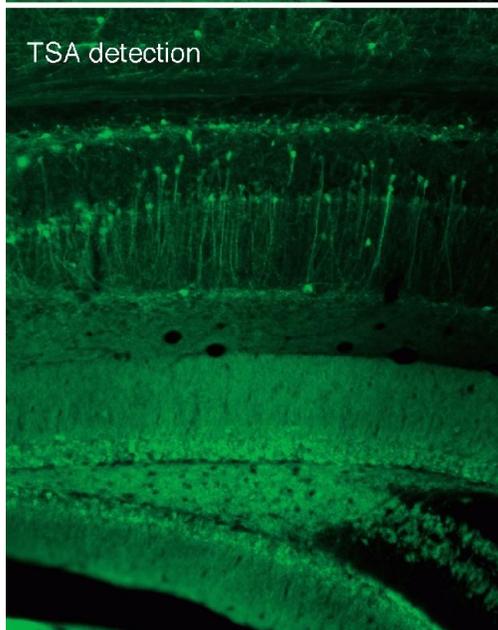
Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11008) in IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 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 $\mu\text{g}/\text{mL}$ Rabbit primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 conjugate (Product # A-11008) was used at a concentration of 4 $\mu\text{g}/\text{mL}$ 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.





Labeled secondary antibody detection



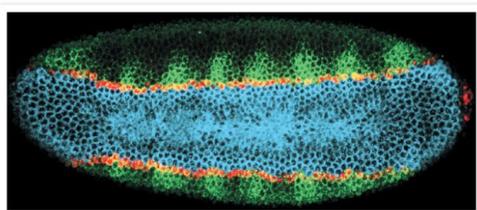
TSA detection

Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11008) in IF

Mice were transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde in phosphate buffer. Thirty μm serial sections were cut in a freezing microtome and transferred to phosphate-buffered saline. Free-floating sections were incubated with 1% hydrogen peroxide to quench endogenous peroxidase activity, blocked in 5% normal goat serum, then stained with a rabbit polyclonal antibody to calbindin D-28K (Chemicon) at a 1:1000 dilution. After washing, sections were incubated with Alexa Fluor[®] 488 goat anti-rabbit IgG antibody (Product # A-11008) at 5 $\mu\text{g}/\text{mL}$ (upper panel) or HRP-goat anti-rabbit IgG antibody at 1 $\mu\text{g}/\text{mL}$, followed by Alexa Fluor[®] 488 tyramide (in TSA Kit #12, T-20922; lower panel). Sections were washed, mounted on slides, coverslipped with ProLong[®] antifade reagent (in Kit P7481) and imaged under identical conditions (10X magnification, 250 millisecond exposure) using a bandpass filter set appropriate for fluorescein (FITC).

Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11008) in IF

Pseudocolored green-fluorescent labeling represents a fluorescein-labeled cRNA probe detected using a rabbit anti-fluorescein/Oregon Green[®] primary antibody (Product # A-889) and an Alexa Fluor[®] 488 dye-labeled anti-rabbit secondary antibody (Product # A-11008). Pseudocolored yellow- and red-fluorescent labeling represents a biotinylated cRNA probe detected using HRP-streptavidin and Alexa Fluor[®] 568 tyramide (TSA Kit #24, Product # T-20934). Pseudocolored blue-fluorescent labeling represents a digoxigenin-labeled cRNA probe detected using a mouse anti-digoxigenin primary antibody in conjunction with an Alexa Fluor[®] 647 dye-labeled anti-mouse secondary antibody (Product # A-21235). The image was contributed by Ethan Bier and David Kosman, University of California, San Diego.



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Immunohistochemistry (20)

Frontiers in cellular neuroscience

Recombinant Adeno-Associated Virus Serotype 6 (rAAV6) Potently and Preferentially Transduces Rat Astrocytes *In vitro* and *In vivo*.

"A-11008 was used in immunohistochemistry to evaluate commercially available adeno-associated virus serotypes for their ability to transduce primary rat astrocytes"

Authors: Schober AL, Gagarkin DA, Chen Y, Gao G, Jacobson L, Mongin AA

Species
Not Applicable

Dilution
1:1000

Year
2018

PloS one

Alpha-Melanocyte Stimulating Hormone Protects against Cytokine-Induced Barrier Damage in Caco-2 Intestinal Epithelial Monolayers.

"A-11008 was used in immunocytochemistry and immunohistochemistry to report that alpha-MSH protects Caco-2 cells against inflammation-induced barrier dysfunction"

Authors: Váradi J, Harazin A, Fenyvesi F, Réti-Nagy K, Gogolák P, Vámosi G, Bácskay I, Fehér P, Ujhelyi Z, Vasvári G, Róka E, Haines D, Deli MA, Vecsernyés M

Species
Not Applicable

Dilution
2 mg/ml

Year
2017

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Immunohistochemistry (Frozen) (20)

Molecular vision

Reduced phosphoCREB in Müller glia during retinal degeneration in *rd10* mice.

"A11008 was used in immunohistochemistry - frozen section to evaluate the mechanisms of retinal degeneration"

Authors: Dong E, Bachleda A, Xiong Y, Osawa S, Weiss ER

Species
Not Applicable

Dilution
Not Cited

Year
2018

Skeletal muscle

Failed reinnervation in aging skeletal muscle.

"A-11008 was used in immunohistochemistry - frozen section to discuss the age-related presence of denervated myofibers and accelerated muscle atrophy"

Authors: Aare S, Spendiff S, Vuda M, Elkrif D, Perez A, Wu Q, Mayaki D, Hussain SN, Hettwer S, Hepple RT

Species
Not Applicable

Dilution
1:500

Year
2018

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ICC (26)

IHC (P) (8)

Flow (2)

WB (3)

IHC (Free) (1)

ChIP (1)

MISC (472)

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