Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 568
Excitation/Emission Max	579/603 nm
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_10563566

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	1:2,000	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

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 bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. 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.

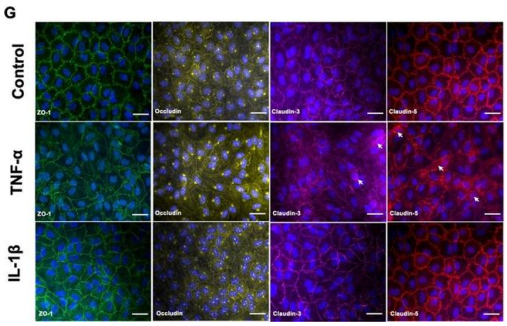
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright,

orange/red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 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 568 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.

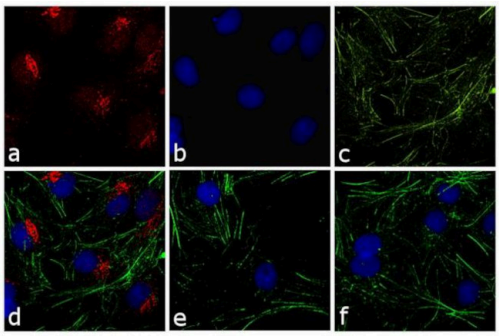
Product will be shipped at Room Temperature.

Product Images For Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568



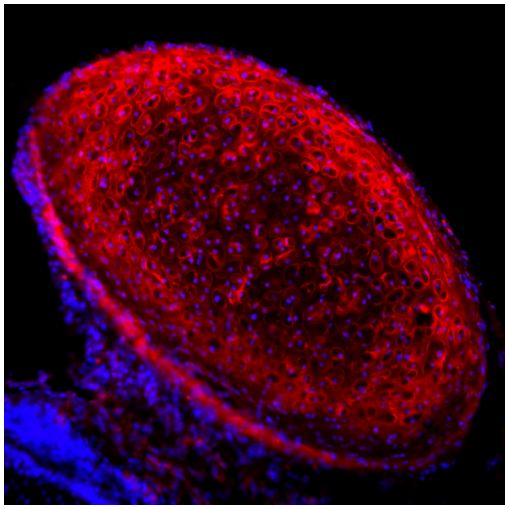
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11036) in ICC/IF

Effects of TNF- or IL-1 treatments on the expression and the localization of junctional protein in BLECs. (A-C) After 24 h of 10 ng·mL1 TNF- treatment, mRNA (A) and protein level (B) of the major protein actors involved in the BBB physical integrity (tight junction protein: ZO-1, tricellulin, occludin, claudin-3, claudin-5; adherent junction protein: VE-cadherin) were determined by RT-qPCR and Western immunoblotting, respectively, compared to the control condition. (C) The immunoblotting images are representative of at least three independent experiments. (D-F) As in the above study, after a treatment of 10 ng·mL1 IL-1 over 24 h, the mRNA (D) and protein levels (E,F) of the same targets were also quantified compared to the control condition. For each of the above graphs (A,B, D,E), the black lines correspond to the control condition value set at 100%. (F) The immunoblotting images are representative of at least three independent experiments. (G) Staining of ZO-1 (green), occludin (yellow), claudin-3 (magenta) and claudin-5 (red) were obtained using immunofluorescence. Each white arrow indicates the presence of stain cytoplasmic dot. Nuclei appear in blue. Scale bar: 50 µm. (H) Claudin-3 and (I) claudin-5 area fraction stainings were quantified in BLECs after exposure to TNF- or IL-1 and were compared to respective control conditions. For all the above graph… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36142143), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11036) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 568 (Product # A-11036) was performed using HepG2 cells stained with alpha-1 antitrypsin Rabbit Polyclonal Primary Antibody (Product # PA5-16661). 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 rabbit primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 568 (Product # A-11036) 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-1 antitrypsin 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) Highly Cross-Adsorbed Secondary Antibody (A-11036) in IHC (F)

Immunohistochemistry analysis of Aggrecan was performed on cryosections of human cartilage. Tissues were blocked in 10% normal goat serum in 1X PBS containing 0.1% Triton X-100 (1X PBS-T) for 1 hour at room temperature (RT). The tissues were labeled with an Aggrecan G3 polyclonal antibody (red, Product # PA1-1745) diluted 1:100 in 3% normal goat serum in 1X PBS-T for 1 hour at RT, followed by detection with a Goat anti-Rabbit IgG (H+L), Alexa Fluor 568 secondary antibody (Product # A-11036) diluted 1:2000 in 3% normal goat serum in 1X PBS-T for 1 hour at RT. Nuclei (blue) were stained with DAPI, included in ProLong Gold Anti-Fade Mountant (Product # P36931). Images were taken on an inverted microscope at 20X magnification. Data courtesy of Dr. Jiyoon Lee at Indiana University School of Medicine.

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1897 References

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