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

Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 488
Excitation/Emission Max	499/520 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_2534122

Applications	Tested Dilution	Publications
Western Blot (WB)	1:3,000-1:5,000	0 Publication
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these rabbit anti-goat IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human and rat serum proteins prior to conjugation. 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.

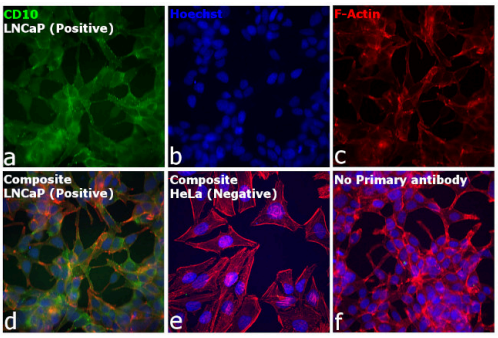
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over 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.

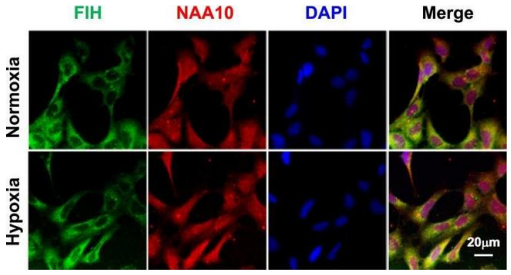
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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.

Product will be shipped at Room Temperature.

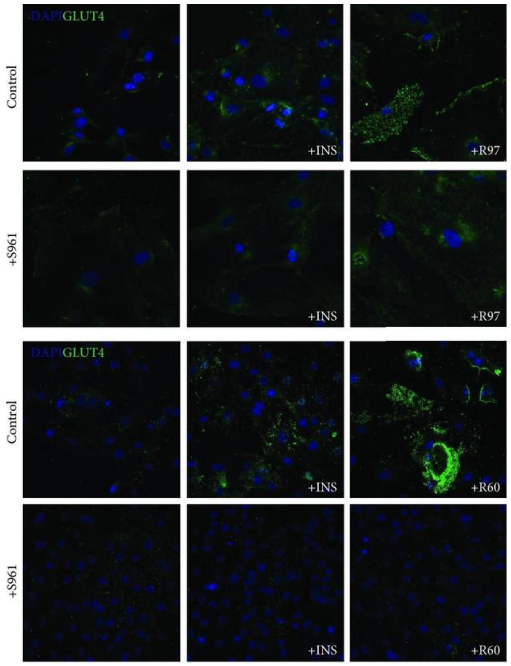
Product Images For Rabbit anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11078) in ICC/IF
Immunofluorescence analysis of Rabbit anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Product # A-11078) was performed using LNCaP (positive model) and HeLa (negative model) cells stained with CD10 Polyclonal Antibody (Product # PA5-47075). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, blocked with 2% BSA for 1 hour and labeled with 1:50 dilution of primary antibody overnight at 4C. Rabbit anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Product # A-11078, 1:2000 dilution) in 0.1% BSA in PBS for 1 hour at room temperature, was used for detection of CD10 on the cell membrane and trans-golgi network (Panel a: green). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor™ 647 Phalloidin (Product # A22287, 1:500) (Panel c: red). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in HeLa (negative model for CD10) due to no primary antibody binding (Panel e). Non-specific staining was not observed with secondary antibody alone (panel f). The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR).



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11078) in ICC/IF
FIH and NAA10 coexist in the cytoplasm and interact with each other. (A) Proteins in HEK293 cell lysates were immunoprecipitated by anti-FIH or anti-NAA10 antibody and immunoblotted using the indicated antibodies. (B) HEK293 cells were transfected with HA-FIH (1 µg/ 60-mm dish) and/or FLAG/SBP-NAA10 (1 µg), and the lysates were subjected to immunoprecipitation using HA or FLAG affinity beads. (C) Recombinant proteins GST-FIH (0.5 µg) and His-NAA10 (0.5 µg) were co-incubated in 20 µl of a binding buffer for 1 h. The mixture was pulled down using glutathione affinity beads and immunoblotted (Top). Protein loading was verified by staining gels with Coomassie brilliant blue (Bottom) (D) HEK293 cells were exposed to normoxia (-) or hypoxia (+) for 24 h. Total lysate (T) was fractionated to cytosolic (C) and nuclear (N) components and the samples were immunoblotted. (E) HEK293 cells were cultured in normoxia or hypoxia for 24 h and subjected to immunofluorescence analysis using anti-FIH and anti-NAA10 antibodies. FIH and NAA10 were visualized with Alexa Fluor 488 (green) and Alexa Fluor 594 (red) conjugated secondary antibodies, respectively. Nuclei were stained with DAPI (blue). (F) Schematic diagram of FIH fragments (top). Each FIH fragment was expressed in HEK293 cells, and the cell lysates were subjected to immunoprecipitation and immunoblotting (bottom). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30237125), licensed under a CC BY license.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11078) in ICC/IF
Effect of R97, R60, or insulin on Glut4 translocation to the plasma membrane in rat cardiac fibroblasts in the presence or absence of the antagonist of the insulin receptor S961. Cells were treated with R97 or R60 (1 mg/mL) for 1 hour or with insulin (100 nM) for 30 min in the presence or absence of 10 nM S961 for 90 min. Glut4 translocation to the plasma membrane was detected using immunofluorescence staining with anti-Glut4 antibody and DAPI nuclear staining as a counterstain. Images were acquired by the Nikon A1 confocal laser scanning microscope (Nikon Instruments, Japan). The results are representative of two independent experiments. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28947927), licensed under a CC BY license.

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199 References

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