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

Product Details	
Size	1 mg
Species Reactivity	Human
Host/Isotype	Goat / 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_2534080

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

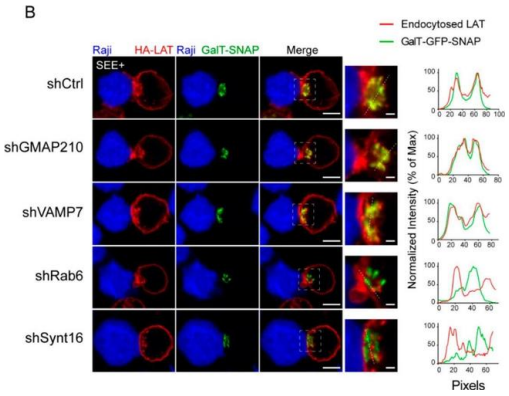
To minimize cross-reactivity, these goat anti-human IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against mouse, rabbit, and bovine serum prior to conjugation. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting 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.

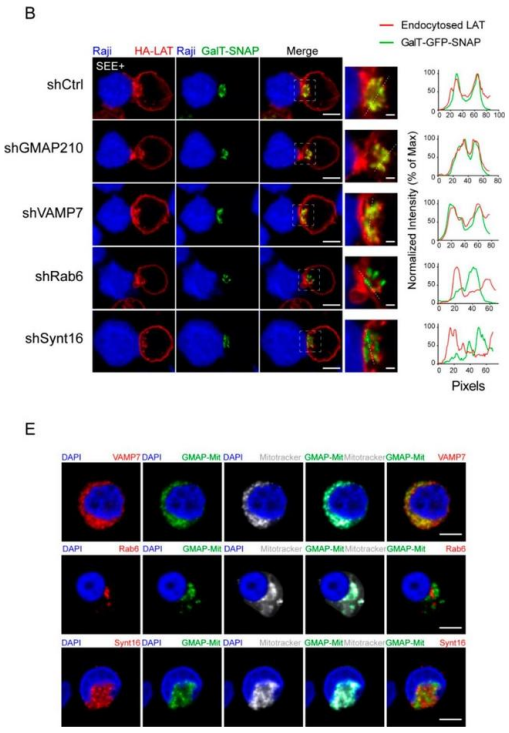
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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 Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11013) in ICC/IF
GMAP210 and VAMP7 do not regulate the retrograde pathway. (A) SNAP trap assay: Jurkat cells expressing GalT-GFP-SNAP and HA-LAT were stained at 4 ° C with anti-HA Ab, washed, and incubated at 4 °C with BG-PEG9-NHS. After washing, cells were activated on slides for 30 min with Raji cells pulsed with SEE. Endocytosed membrane proteins, coupled with BG, covalently bound to the SNAP domain of the GalT-GFP-SNAP protein getting trapped in the Golgi. (B) Confocal images of SNAP trap assay in Jurkat cells expressing GalT-GFP-SNAP, HA-LAT and control (Ctrl) or GMAP210/VAMP7/Rab6/Synt16 specific shRNA. Labelings were performed using anti-mouse Ig (Alexa Fluor 568) to label the anti-HA Ab and anti-GFP to label the GalT-GFP-SNAP. Dashed square points to the inset localization containing the Golgi apparatus. Dashed line indicates where HA-LAT (red) and GalT-GFP-SNAP fluorescence signal intensity was measured for the plot. Images show the z-projection of summed slices from three stacks covering the Golgi apparatus. Scale bars = 5 µm. Inset scale bars = 1 µm. (C) Quantification of Mander's overlapping coefficient of GalT-GFP-SNAP over HA-LAT. (D), Mitochondrial trapping assay: Jurkat cells expressing a chimeric GFP tagged GMAP210 protein with the C-terminal hydrophobic anchor of ActA can capture vesicles interacting with GMAP210 in the mitochondria. (E) Confocal images showing the localization of VAMP7, Rab6 or Sy… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33572370), licensed under a CC BY license.



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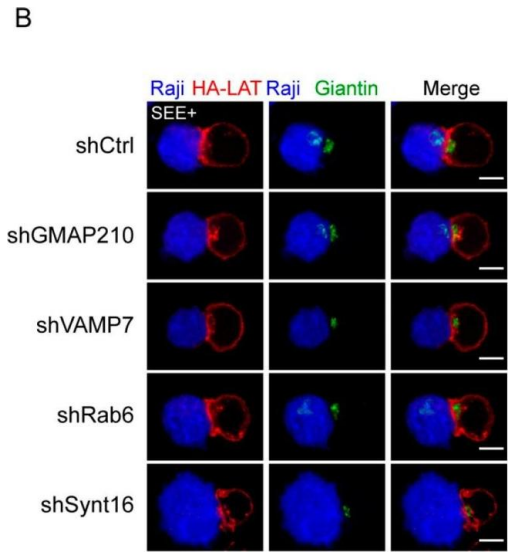

Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11013) in ICC/IF
Retrograde and anterograde pathways regulate the recruitment of endocytic pool of LAT at the IS. (A) Confocal images of HA-LAT-Jurkat cells transduced with nontargeting control shRNA (shRNA Ctrl) or GMAP210/VAMP7/Rab6/Synt16-targeting shRNAs, fixed before (left panel, 0 h) or after endocytosed LAT assay (right panel, 4 h) and stained for endocytosed LAT (HA-LAT, red) and the Golgi apparatus marker Giantin (green). (B) Confocal images of conjugates between HA-LAT-Jurkat cells (after endocytosed LAT assay) expressing control (Ctrl) or GMAP210/VAMP7/Rab6/Synt16 specific shRNA, and SEE pulsed Raji cells (blue), labeled with anti-Giantin (green) and anti-mouse 568 (red). (C) Average cell representation and (D) quantification of the enrichment of endocytosed LAT at the immune synapse (depicted by the dotted white line) in control or GMAP210 /VAMP7/Rab6/Synt16 silenced cells incubated with unpulsed (, unactivated state) or SEE pulsed (+, immune synapse formation) Raji cells for 30 min. n = number of cells constituting the mean image. Horizontal lines represent the median. Images in (A,B) show the z-projection of summed slices from three stacks covering the Golgi apparatus in T cells. Scale bars = 5 µm. Two-way ANOVA ** $p < 0.01$, **** $p < 0.0001$, ns: nonsignificant. Data and images are from two independent experiments in (A) and from three independent experiments (B-D) Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33572370), licensed under a CC BY license.

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384 References

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