

Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 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_2535850

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))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	-
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 may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 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 555 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.

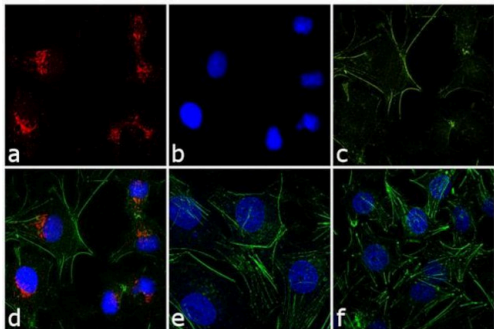
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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™ 555

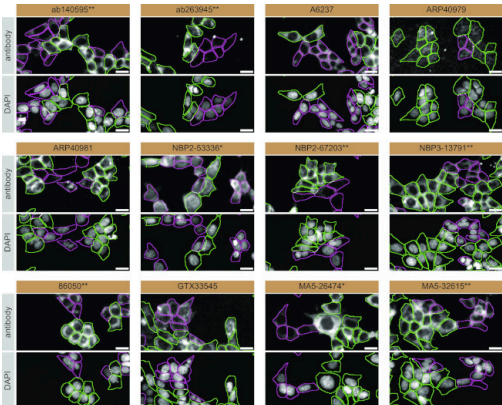
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21429) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555 (Product # A-21429) 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 555 (Product # A-21429) 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.



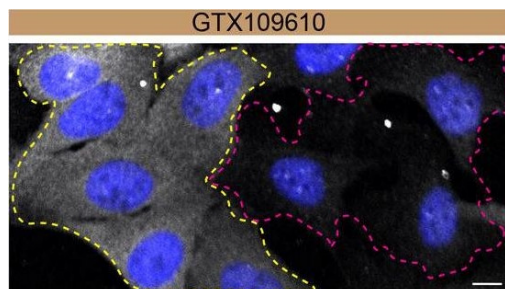
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21429) in ICC/IF

TIA1 antibody screening by immunofluorescence. HAP1 WT and TIA1 KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio in a 96-well plate with optically clear flat-bottom. Cells were stained with the indicated TIA1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibody dilutions were chosen according to the recommendations of the antibody supplier. Exceptions were given to antibodies ab140595**, A6237, NBP2-67203**, and GTX33545, which were titrated to their respective concentrations found bellow, as the signals were too weak when following the suppliers' recommendations. When the concentrations were not indicated by the supplier, which was the case for antibodies ab263945**, ARP40979, ARP40981, and NBP3-13791** we tested antibodies at 1/300, 1/500, 1/500 and 1/200, respectively. At these concentrations, the signal from each antibody was in the range of detection of the m - Image collected and cropped by CiteAb under a CC-BY license from the following publication: A guide to sele... Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/38638178>), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21429) in ICC/IF

Charged multivesicular body protein 2b antibody screening by immunofluorescence. U2OS WT and CHMP2B KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Charged multivesicular body protein 2b antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilutions were chosen according to the recommendations of the antibody supplier. Exceptions were given to antibodies ab157208** and A13410, which were titrated to 1/1000 and 1/800, respectively, as the signals were too weak when following the suppliers' recommendations. When the concentration was not indicated by the supplier, which was the case for antibodies MA5-21791* and 76173*, we tested antibodies at 1/500. At this concentration, the signal from each antibody was in the range of detection of the microscope used. Antibody dilution used: MAB7509* at 1/500; MA5-21591* at 1/500; MA5-36184** at 1/1000; ab1 - Image collected and cropped by CiteAb under a CC-BY license from the following publication: The identification of high-perf... Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/37635943>), licensed under a CC BY license.



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957 References

A guide to selecting high-performing antibodies for ADNP (UniProt ID: Q9H2P0) for use in western blot, immunoprecipitation, and immunofluorescence. F1000Res (2025)

A guide to selecting high-performing antibodies for VAPB (UniProt ID: O95292) for use in western blot, immunoprecipitation, and immunofluorescence. F1000Res (2025)

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