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

Product Details	
Size	1 mg
Species Reactivity	Mouse
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_141780

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	Assay-dependent	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

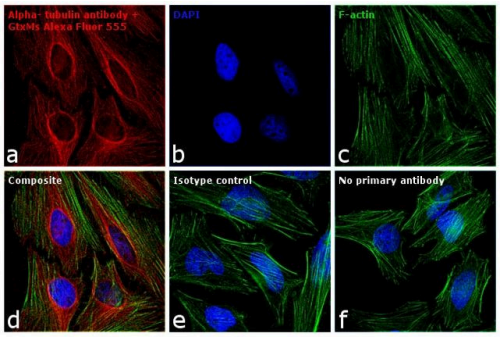
To minimize cross-reactivity, these goat anti-mouse IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against bovine IgG, goat IgG, rabbit IgG, rat IgG, human IgG, and human serum. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the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

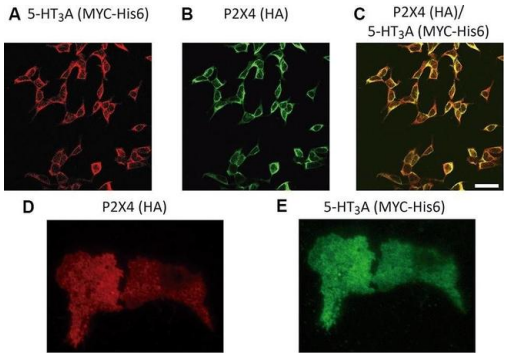
Product will be shipped at Room Temperature.

Product Images For Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



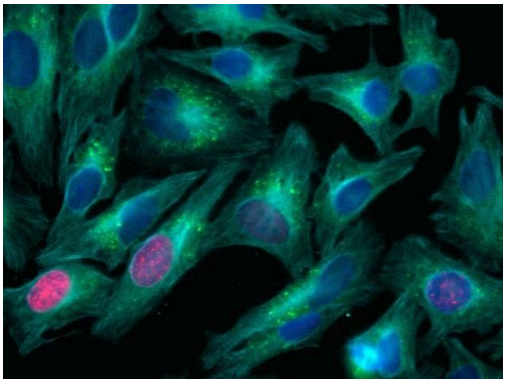
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424) in ICC /IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal Antibody (Product # A11126). 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 primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 (Product # A-21424) 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 Tubulin 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.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424) in ICC /IF

Expression and purification of P2X4/5-HT3A receptor complexes. (A,B) Detection of co-expressed 5-HT3A (A) and P2X4 (B) receptors by confocal immunofluorescence in tsA201 cells via anti-MYC (1:250) and anti-HA (1:250) antibodies, followed by their corresponding secondary antibodies conjugated to Alexa Fluor®555 and Alexa Fluor®488, respectively. The merge of panels (A,B) is shown in (C), where scale bar represents 30 µm. (D,E) Analysis by total internal reflection fluorescence (TIRF) microscopy of co-expressed P2X4 (D) and 5-HT3A (E) receptors individually and merge images (F) in tsA201 cells via anti-HA (1:250) and anti-MYC (1:250) antibodies, followed by their corresponding secondary antibodies conjugated to Alexa Fluor®555 and Alexa Fluor®488, respectively. Scale bar in (F) represents 3 µm. (G) Colocalization analysis of the P2X4/5-HT3A receptor complexes in tsA201 cells (n = 26 from two independent experiments). (H,I) P2X4 receptor expression analysis in tsA201 cells through TIRF (H, n = 26 for P2X4/NRP and P2X4/5-HT3A transfections from two independent experiments) and epifluorescence (I, n = 273 for P2X4 and 254 for P2X4/5-HT3A transfections from two independent experiments) measurements. Non-related plasmid (NRP) corresponds to pDNA IRES-GFP (0.5 µg each well) used in the same amount as the other receptor plasmids. Data are shown as mean ± SE. *p < 0.05 vs. P2X4/NRP expression by… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32431598), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21424) in ICC /IF

HeLa cells were treated with 30 µM chloroquine and cultured overnight. The following day, the cells were fed 10 µM EdU under regular growth conditions for one hour and then fixed and permeabilized. EdU was used to was visualize proliferating cells using The Click-iT® EdU Alexa Fluor® 488 Imaging kit (pink). Cells were counter stained with 0.5 µg/mL anti-LC3B with a goat anti rabbit Alexa Fluor® 647 secondary (Green), mouse anti alpha tubulin with a goat anti mouse Alexa Fluor® 555 secondary (Cyan) and 1 µg/mL Hoechst 33342 (Blue). Cells were imaged on a Molecular Devices ImageXpress High content imager.

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736 References

HDAC2 counteracts vascular calcification by activating autophagy in chronic kidney disease. FASEB J (2024)

MARC-3, a membrane-associated ubiquitin ligase, is required for fast polyspermy block in Caenorhabditis elegans. Nat Commun (2024)

Golgi-to-ER retrograde transport prevents premature differentiation of Drosophila type II neuroblasts via Notch-signal-sending daughter cells. iScience (2024)

RREB1 regulates neuronal proteostasis and the microtubule network. Sci Adv (2024)

SH3RF2 contributes to cisplatin resistance in ovarian cancer cells by promoting RBPMS degradation. Commun Biol (2024)