



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

Product Details	
Size	1 mg
Species Reactivity	Rat
Host/Isotype	Goat / IgG
Class	Polyclonal
Туре	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_2535855

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	1-10 μg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 μg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these goat anti-rat IgG (H+L) whole secondary antibodies have been affinity purified and crossadsorbed against mouse IgG, mouse serum, and human 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 crossreactive 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.

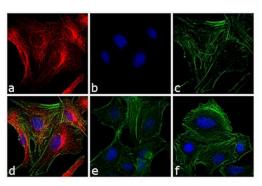
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive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

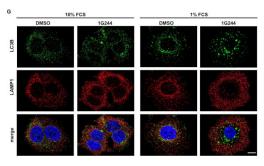
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

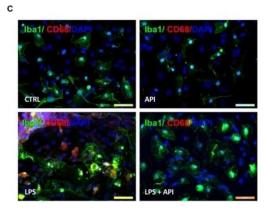
Product Images For Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21434) in ICC/IF Immunofluorescence analysis of Goat anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor® 555 was performed using A549 cells stained with alpha Tubulin (YL1/2) Rat Monoclonal Antibody (Product # MA1-80017). 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 2µg/mL Rat primary antibody for 3 hours at room temperature. Goat anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor® 555 (Product # A-21434) was used at a concentration of 2µg/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.



Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21434) in ICC/IF DPP8/9 inhibition separates lysosomes and autophagosomes in MCF-7 cells. (A-C) (A) p62/SQSTM1, (B) LC3-I/LC3-II, and (C) LAMP1 protein expressions in MCF-7 cells ± 1G244 and 10% or 1% FCS via Western blot. Protein expressions of p62/SQSTM1 and LAMP1 were normalized to TUBA (n = 5). (D-F) Relative (D) p62/SQSTM1, (E) LC3B, and (F) LAMP1 mRNA expressions normalized to ACTB in MCF-7 cells ± 1G244 and 10% or 1% FCS via qRT-PCR (n 3). (G) Immunofluorescence of LC3B (green) and LAMP1 (red) in MCF-7 cells ± 1G244 and 10% or 1% FCS (DNA: Hoechst (blue); n = 3). Scale bar: 10 μm. Bar charts show all data points with means + SEMs and p-values calculated via paired-sample t-test. - Image collected and cropped by CiteAb under a CC-BY license from the following publication: Dipeptidyl-Aminopeptidases 8 and 9 Regulate Autophagy and Tamoxifen Response in Breast Cancer Cells. <i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</ti>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</i>Cells</ti>Cells</ti>Cells</ti>Cells



Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21434) in ICC/IF Apigenin modulates microglial activation profile. Effects of apigenin (API, 1 M) treatment on the activation of microglia after LPS (A-E, 1 g/mL), or IL-1 (F, 10 ng /mL) inflammatory stimuli in glia/neurons co-cultures. Proliferation and changes in morphology, both features of microglial activation were analyzed. (A,B) Immunocytochemistry for ionized calcium-binding adapter molecule 1 (lba-1, green), specific of microglia, associated with bromodeoxyuridine (BrdU; red), that marker cells in proliferation was performed in cultures submitted to inflammatory stimuli with LPS. Graphs (B,D) represents cell population labeled for both lba-1 and BrdU; data a represented as mean of percentage ± standard deviation of immunofluorescence labeling of Iba-1 and BrdU cells (C) Immunocytochemistry for Iba-1 (green), associated with CD68 (red), marker of activated microglia /macrophages in a proinflammatory profile was performed after 24 h treatments. (D,E) The graphs represent the total population of Iba-1 positive cells (microglia) and the population of double-labeled Iba-1 +/CD68 + microglia. (F) Immunocytochemistry for the cytoskeletal protein lba-1 (red), was also performed in cultures submitted to inflammatory stimuli with IL-1; control cultures were treated with DMSO (0.01%) and nuclear chromatin was stained with DAPI (blue); Obj. 40x, scale bar: 50 m.; *represents sta... Image collected and cropped by CiteAb from the following publication (https://www.frontiersin.org/article/10.3389 /fnagi.2020.00119/full), licensed under a CC BY license.

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□ 772 References

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