

Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 633
Excitation/Emission Max	631/650 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_2535731

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 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-rabbit IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine 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 may be the presence of endogenous immunoglobulins.

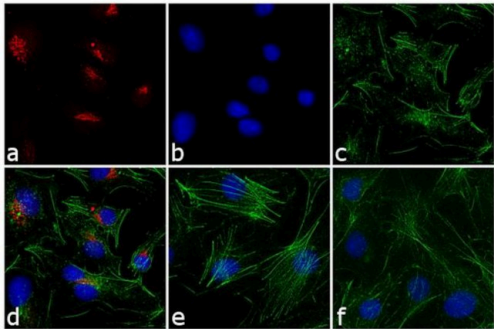
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 633 dye is a bright, far-red-fluorescent dye with excitation ideally suited to the 633 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 633 dye is pH-insensitive 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 633 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.

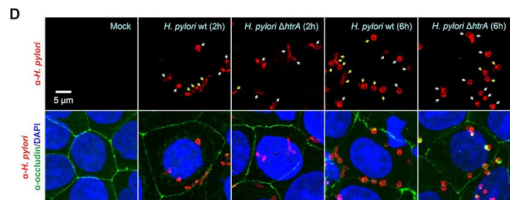
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

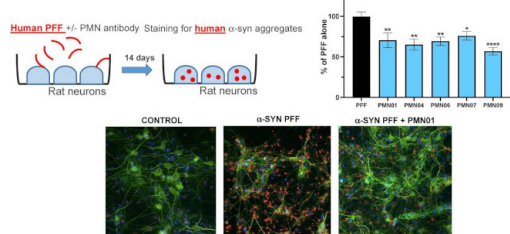
Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21070) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 633 was performed using HepG2 cells stained with alpha-1 antitrypsin Rabbit Polyclonal Primary Antibody (Product # PA5-16661). The cells were 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 rabbit primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 633 (Product # A-21070) 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) Cross-Adsorbed Secondary Antibody (A-21070) in ICC/IF
Infection of the gastric epithelial MNK-28 cells by *H. pylori* and RNA-seq analysis. (A) Knockout of the *HtrA* protease gene in *H. pylori* strain N6 confirmed by Western blotting using -*HtrA* and -*FlaA* antibodies. (B) Quantification of bacterial cells adhered to MNK-28 monolayers based on microscopy analysis. Differences in number of adherent *H. pylori* wt and *H. pylori* *htrA* cells were non-significant (n.s.) after either 2 or 6 h of infection. (C) Ratio of *H. pylori* wt and *H. pylori* *htrA* cells showing non-junctional and junctional localization. Significant differences in ratios were defined as (p < 0.05). n.s. - non-significant. (D) Confocal microscopy of MNK-28 cell monolayers infected with *H. pylori* N6 wt or *htrA* mutant for 2 or 6 h. The samples were stained with -*H. pylori*, -occludin and DAPI to visualize bacterial cells (red), cellular tight junctions (green) and nuclei (blue), respectively. Arrows indicate bacterial cells at junctional (yellow arrows) or non-junctional (white) localization. (E) Principal component analysis (PCA) of rlog transformed read counts across all replicates in uninfected mock MNK-28 cells, MNK-28 infected with *H. pylori* wt or *htrA* mutant for 2 or 6 h. The most variation (88%) is explained by the principal component 1 (PC1), showing dependence on image collection and cropping by CiteAb under a CC-BY license from the following publication: Early ... Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/37193047), licensed under a CC BY license.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21070) in ICC/IF
mAb inhibition of PFF uptake and intracellular aggregate formation. Cultures of primary rat hippocampal neurons were exposed to sonicated human PFF (1 µg/mL) without or with mAbs (0.05 µg/mL, except for PMN09 at 0.25 µg/mL). Cultures were stained 14 days later for neuronal marker MAP2 (green), aggregates of human I⁺syn (red) and cell nuclei (blue). Results are expressed as a percentage of the human I⁺syn staining area with PFF alone and show the mean ± SEM of 6 replicate cultures. A global analysis of the data was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001 vs. PFF. - Image collected and cropped by CiteAb under a CC-BY license from the following publication: Rational Generation of Monoclonal Antibodies Selective for Pathogenic Forms of Alpha-Synuclein. *Biomedicine* (2022) Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36140270), licensed under a CC BY license.



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