Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 647
Excitation/Emission Max	650/671 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_2535813

Applications	Tested Dilution	Publications
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
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Dot blot (DB)	-	0 Publication
Immunomicroscopy (IM)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

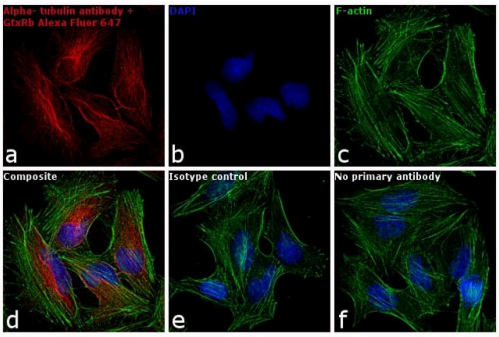
To minimize cross-reactivity, these goat anti-rabbit IgG whole antibodies have been cross-adsorbed against bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less 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 are may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 647 dye is a near-infrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 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 647 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.

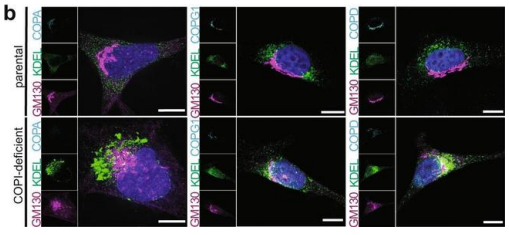
Product will be shipped at Room Temperature.

Product Images For Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647



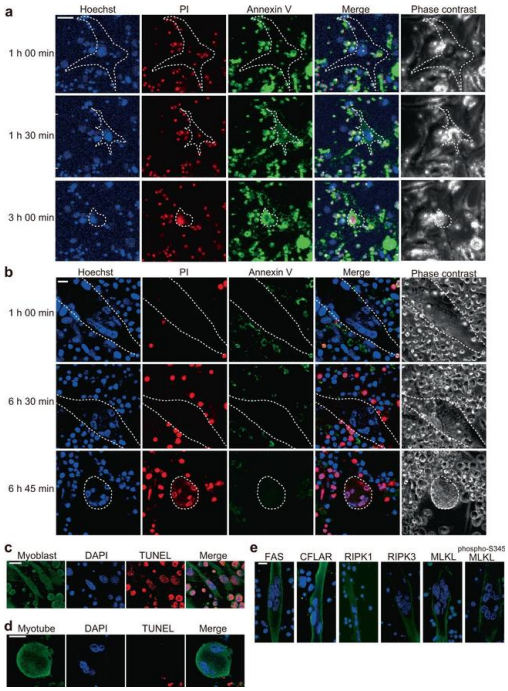
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21245) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 647 conjugate was performed using HeLa cells stained with alpha Tubulin Rabbit Polyclonal Antibody (Product # PA516891) 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-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 647 conjugate (Product # A-21245) was used at a concentration of 2 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.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21245) in ICC/IF

cGAS/STING pathway activation is caused by general deficiency in COPI-mediated retrograde trafficking.a Schematic illustration of coatomer complexes COPII (green) and COPI (blue) mediating intracellular trafficking between endoplasmic reticulum (ER) and Golgi compartments. The COPII complex mediates anterograde transport from ER to Golgi, whereas COPI mediates retrograde transport from Golgi to ER as well as within cis-Golgi compartments. COPI vesicle formation occurs at the Golgi membrane through interaction of 7 subunits: (COPA), (COPB1), ' (COPB2), (COPD), (COPE), (COPG), (COPZ). After vesicle budding, the coatomer coats are shed off and released into the cytoplasm to allow vesicle fusion at the target membrane2. b Representative IF images of parental, COPAdeficient, COPG1deficient and COPDdeficient HeLa cells co-stained for KDEL (green), GM130 (magenta), indicated COPI subunit (cyan) and DAPI (blue). Images are presented as maximum intensity projection. Scale bar 10 µm. c Quantification of IF co-localization of KDEL and GM130 signal in COPI subunit-deficient HeLa cells shown in (b). Results are quantified as percentage area of KDEL localization (ER-specific retention signal) inside cis-Golgi (GM130). Each dot represents one single cell. Parental cells stained for different subunits were combined for quantification analysis. Statistical significance was as… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35484149), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21245) in ICC/IF

Different pattern of cell death between myoblasts and myotubes in the co-culture with CTLs.a, b The representative confocal z-stack images of time-lapse analysis of H2KbOVA-myoblasts (a) or H2KbOVA-myotubes (b) co-cultured with OT-I CTLs in the presence of Annexin V (green) and PI (red). Nuclei of the cells were stained with Hoechst 33342 (blue). Images were taken at the indicated times from the starting of the co-culture. The cells surrounded by dotted line indicate dying myoblast and myotube. Scale bars indicate 20 µm. Representative data of three independent experiments are shown. c, d The TUNEL staining (red) of H2KbOVA-myoblasts (c) or H2KbOVA-myotubes (d) co-cultured with OT-I CTLs for 4 or 16 h, respectively. The myoblasts and myotubes were pre-labelled with CellTracker (green). Confocal z-stack images are shown. Scale bars indicate 20 µm. Representative data of two independent experiments are shown. e The immunofluorescence staining of FAS, CFLAR, RIPK1, RIPK3, MLKL, and phosphorylated MLKL at S345 (phospho-S345 MLKL; green) in H2KbOVA-myotubes co-cultured with OT-I CTLs for 8 h. Nuclei were counterstained with DAPI (blue). Scale bar indicates 10 µm. Representative data of three independent experiments are shown. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35013338), licensed under a CC BY license.

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1727 References

Whole-body cellular mapping in mouse using standard IgG antibodies. Nat Biotechnol (2024)

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