Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 546
Excitation/Emission Max	561/572 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_2534093

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	1-10 µg/mL	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	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 are may be the presence of endogenous immunoglobulins.

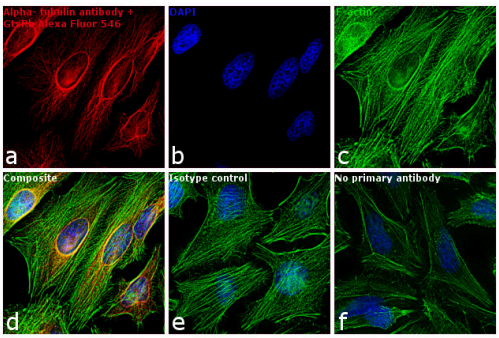
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 546 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 546 nm laser line. For stable signal generation in imaging and flow

cytometry, Alexa Fluor 546 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 546 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.

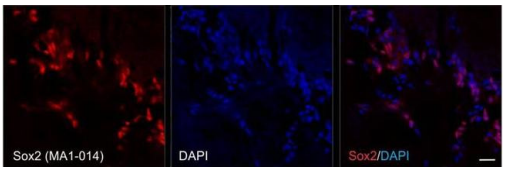
Product will be shipped at Room Temperature.

Product Images For Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546



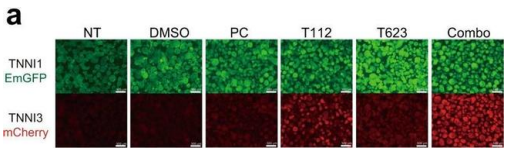
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11035) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 546 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® 546 conjugate (Product # A-11035) 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.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11035) in IHC (F)

Immunofluorescent analysis of Phospho-RAD17 pSer646 showing staining in the nucleus of HCT116 cells. HCT116 cells were fixed in 4% paraformaldehyde at RT for 15 min and stained using a Phospho-RAD17 pSer646 polyclonal antibody (Product # PA5-34970) diluted at 1:500. Blue: Hoechst 33342 staining. Scale bar = 10 µm.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11035) in ICC/IF

T112 and T623 enhanced the cardiac maturation of hiPSC-CMs.a Fluorescence images of day-16-derived EBs for TNNI1EmGFP and TNNI3mCherry expression. Scale bars: 500 µm. NT no treatment, PC positive control. b Flow cytometric analysis of day-16-derived cells for TNNI3mCherry expression. n = 3 independent experiments per group. Data are the mean ± SEM; ****P < 0.0001 compared to DMSO using one-way ANOVA followed by Dunnett's test. NT no treatment, PC positive control. c Principal-component analysis (PCA) in correlation with the maturation of CMs for more than 58,000 genes expressed in the four groups. Data are shown as average PCA for each group. n = 3 independent experiments per group. d Sample correlation matrix resulting from the consensus clustering analysis of mRNA data and showing transcriptomic differences among treatments. Data are shown as averages for each group. n = 3 independent experiments per group. e Volcano plots for DE genes in accordance with the cardiac maturation phenotype. f Summary of upregulated DE genes in either T623/T112-treated CMs or Combo-treated CMs compared to DMSO. The number of upregulated genes (at least 2-fold) is depicted. Left: 344 unique elements detected in the T623-treated CMs compared to DMSO. Middle: 947 unique elements detected in the T112-treated CMs compared to DMSO. Right: 1254 unique elements detected in the Combo-treated CMs compared to DMSO. g Analysis of the top… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34155205), licensed under a CC BY license.

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1221 References

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