Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 594
Excitation/Emission Max	590/618 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_2534091

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	Assay-dependent	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 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-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.

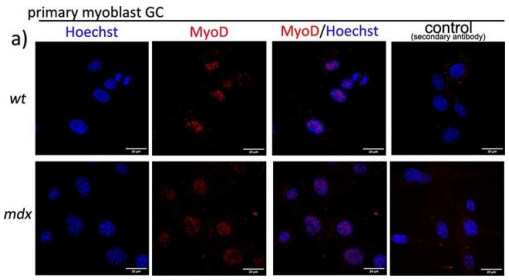
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 594 dye is a bright, red-fluorescent dye with excitation ideally suited to the 594 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 594 dye is pH-insensitive over 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 594 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.

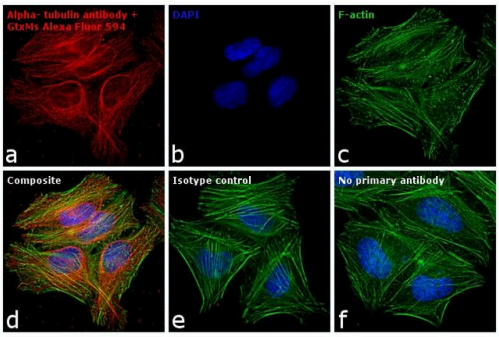
Product will be shipped at Room Temperature.

Product Images For Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594



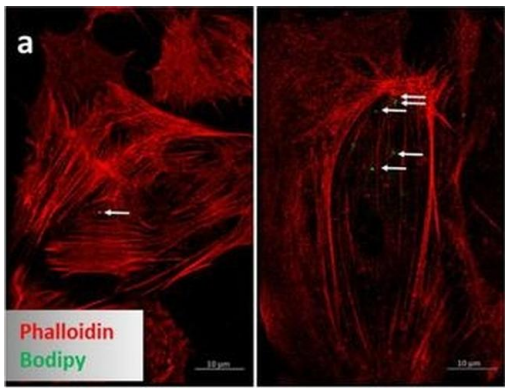
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11032) in ICC/IF

The purity of myoblasts isolated from the gastrocnemius muscle.(a) Example MyoD staining (red signal) and Hoechst nuclear labelling (blue) in primary myoblast cultures isolated from mdx and wild-type gastrocnemii. Representative immunofluorescent image from three tiles are presented and the negative control (no primary antibody) is also shown. (Size bar = 20 µm). (b) Graph showing percentage of cells positive for MyoD from the total number of cells identified by the nuclei labelling with Hoechst enumerated using the counter cells function in the ImageJ software. The purity of wt and mdx myoblast cultures was between 93% and 100%. Error bars represent the mean number of cells with SD for three tile images and the difference is not statistically significant. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36164827), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11032) in ICC/IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 594 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® 594 (Product # A-11032) 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.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11032) in ICC/IF

Monocyte/platelet EVs activate HUVEC in vitro. [EVs were collected from isolated monocyte/platelet aggregates, isolated platelets or THP1 cells incubated with vehicle (V) or TNF- (50 ng/ml) for 60 min, in presence or absence of Iloprost (2 µM; PGI2) for 60 min. HUVEC were incubated with the different EV sets (10 × 106/ml) overnight. Cells were stained for flow cytomentry analysis and supernatants collected and analysed for cytokine release. (a) Confocal images of the uptake by HUVEC stained with Phalloidin (red) after 24 h of BODIPY labelled EV isolated from TNF- stimulated cells (white arrows, left panel) or from unstimulated cells (white arrow, right panel). (b) IL-6 levels by ELISA. (c-d) Quantification of ICAM-1 and VCAM-1 expression (MFI units) in HUVEC treated with different subsets of monocyte/platelet derived EVs, platelet-derived EVs, THP1-derived EVs. (*P < 0.05, **P < 0.01, ***P < 0.001; one-way ANOVA post Bonferroni test, mean ± SEM of n = 3-6 cell preparation incubated with distinct EV preparations from different donor cells)] Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33936566), licensed under a CC BY license.

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1842 References

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