Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 488
Excitation/Emission Max	499/520 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_2534088

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	Assay-dependent	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication
Not applicable (N/A)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, the goat anti-mouse IgG whole antibodies have been highly 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. Further passages through additional columns result in 'highly cross-adsorbed' preparations of secondary antibody. The benefits of these extra steps are apparent in multiplexing/multicolor-staining experiments 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.

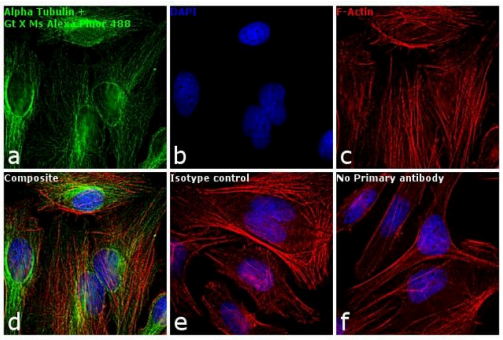
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow

cytometry, Alexa Fluor 488 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 488 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.

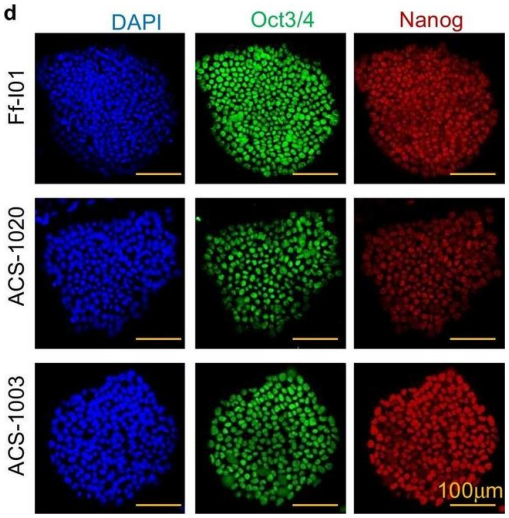
Product will be shipped at Room Temperature.

Product Images For Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



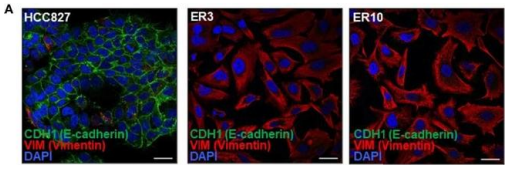
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11029) in ICC/IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 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 Mouse primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor® 488 conjugate (A-11029) was used at a concentration of 1 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: green). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Rhodamine Phalloidin (Product # R415, 1:300) (Panel c: red). 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-11029) in ICC/IF

ON2 medium sustains the prolonged expansion of different hiPSC lines. a Ff-I01, ACS-1020, and ACS-1003 hiPSC clones after long-term expansion of iMatrix-511 in the ON2 medium. Scale bar, 200 µm. b Comparison of doubling times for Ff-I01, ACS-1020, and ACS-1003 hiPSCs cultured in ON2 (passage number = 12-15, n.s. no significant difference; data are mean ± SD) (Ff-I01: 23.09 ± 2.88; ACS-1020: 24.78 ± 3.02; ACS-1003: 25.28 ± 2.21. c FACS analysis of pluripotent marker-positive cells. Light-gray solid histograms show the isotype control populations, and color hollow histograms show the stained populations, respectively. The percentages of maker-positive cells are presented in each graph. Cell events were normalized to mode. d Immunofluorescence staining of pluripotency markers in hiPSCs. Green: Oct3/4; red: Nanog; blue: DAPI. Scale bar, 100 µm. e qRT-PCR analysis of pluripotent gene expression levels of OCT4, NANOG, SOX2, and KLF4 in three hiPSCs sustained in ON2. Fold expression was compared to that in 253G1 hiPSC cell lines in ON2. (n = 3 biological independent sample, n.s. no significant difference; data are mean ± SD) Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35658933), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-11029) in ICC/IF

Resistance to first- and second-generation EGFR inhibitors is associated with features of EMT. (A) Immunocytochemistry of HCC827 parental cells and the erlotinib-resistant clones ER3 and ER10 for the epithelial marker CDH1 (E-cadherin) and the mesenchymal marker VIM (vimentin) to examine markers of epithelial plasticity upon acquired drug resistance. Counterstain by DAPI. Scalebar = 30 µm. (B) TUBA1A (alpha-tubulin) immunocytochemistry of the cells described in (A) were applied to reveal the phenotypic shift in cell morphology. Counterstain by DAPI. Scalebar = 30 µm. (C) Western blots were prepared with lysates from the HCC827 parental cells and the erlotinib-resistant clones ER3, ER10, ER20, and ER30 H1975 parental cells and the clones COR1-1 and COR10-1 resistant to the second-generation EGFR inhibitor rociletinib. Immunodetection of epithelial marker CDH1 (E-cadherin) (135 kDa), mesenchymal markers CDH2 (N-cadherin) (135 kDa), VIM (vimentin) (54 kDa). Western blot analysis was repeated n = 3 times, and a representative experiment is presented in the figure. (D) Quantification of the western blot presented in (C) normalized against total protein presented in Supplementary Figure 1A (VIM and CDH1) and B (CDH2). Fold change values for the resistant clones ER3 and ER10 relative to their parental cell line HCC827 (E) Expression of transcripts encoding CDH1, CDH2, VIM, assessed by RT-qPCR on cDNA prepared from HCC8… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35530312), licensed under a CC BY license.

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5265 References

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