F(ab')2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594

Product Details	
Size	500 µg
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_2534087

Applications	Tested Dilution	Publications
Western Blot (WB)	1:2,500-1:5,000	-
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	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-mouse IgG (H+L) divalent F(ab')2 secondary antibodies have been affinity purified and cross-adsorbed against human IgG and 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.

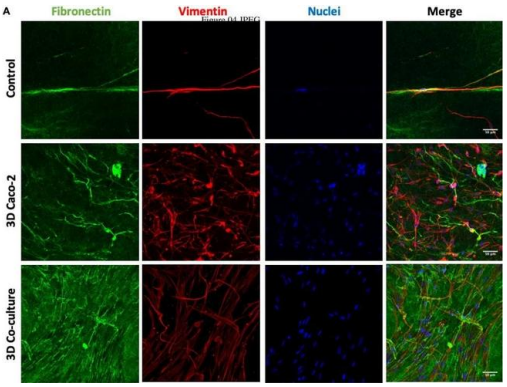
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 594 dye is a bright, red-fluorescent dye with excitation ideally suited to the 594 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 594 dye is pH-insensitive over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

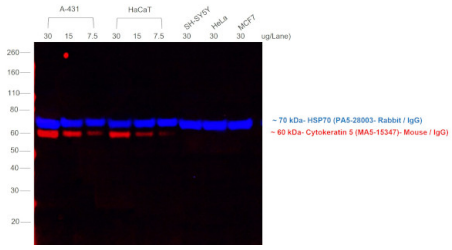
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

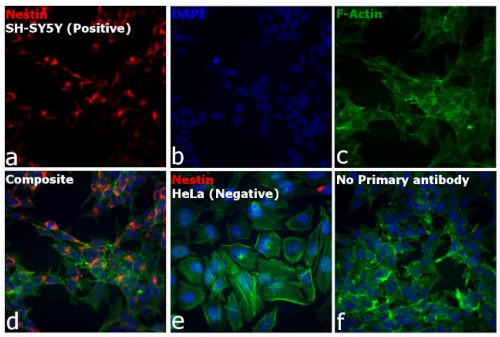
Product Images For F(ab')2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11020) in ICC/IF
Human intestinal fibroblast (HIF) morphology and (A) fibronectin and (B) laminin deposition by HIFs in collagen hydrogels, during the time in culture, with and without epithelial cells on top. The control refers to HIF embedded in the collagen layer alone, and the 3D Caco-2 and 3D Coculture refer to the addition of Caco-2 and Caco-2 + HT29-MTX on top of this layer, respectively. Pictures show maximal projections of several stacks of the gels. Fibronectin and laminin were labeled with Alexa-Fluor 488 (green), and vimentin was labeled with Alexa-Fluor 594 (red). The nucleus was counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Image collected and cropped by CiteAb from the following publication (https://www.frontiersin.org/article/10.3389/fbioe.2020.524018/full), licensed under a CC BY license.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11020) in WB
Multiplexed fluorescent western blot was performed using F(ab)2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Product # A-11020). Whole cell extracts of A-431 (Lane 1, 2, 3), HaCaT (Lane 4, 5, 6), SH-SY5Y (Lane 7), HeLa (Lane 8), and MCF7 (Lane 9) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with Cytokeratin 5 Monoclonal Antibody (3E2F1) (Product # MA5-15347), and HSP70 Polyclonal Antibody (Product # PA5-28003). Secondary antibodies (Product # A-11020, 1:5000 dilution), and (Product # SA5-35571, 1:20000 dilution) were used for detection of Cytokeratin 5, and HSP70 respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # A-11020) specifically detects the mouse primary antibody.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11020) in ICC/IF
Immunofluorescence analysis of F(ab)2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594, (Product # A-11020) was performed using SH-SY5Y (positive model) and HeLa (negative model) cells stained with Nestin Monoclonal Antibody (10C2), eBioscience™ (Product # 14-9843-80). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 2% BSA for 1 hour and labeled with 1:500 dilution primary antibody overnight at 4C. F(ab)2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (Product # A-11020, 1:2000 dilution) in 0.1% BSA in PBS for 1 hour at room temperature, was used for detection of Nestin in the cytoskeleton (Panel a: Red). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:500) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in HeLa (negative model for Nestin) due to no primary antibody binding (Panel e). Non-specific staining was not observed with secondary antibody alone (panel f). The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR).

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249 References

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