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

Product Details	
Size	500 µg
Species Reactivity	Rabbit
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_2534116

Applications	Tested Dilution	Publications
Western Blot (WB)	1:2,500-1:5,000	-
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1:500-1:2,000	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these goat anti-rabbit IgG (H+L) divalent F(ab')2 secondary antibodies have been affinity purified and cross-adsorbed against pooled human serum, mouse serum, mouse plasmacytoma/hybridoma proteins, and purified human paraproteins. 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.

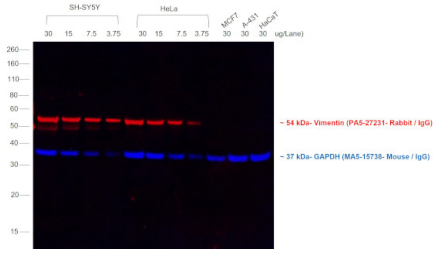
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 594 dye is a bright, red-fluorescent dye with excitation ideally suited to the 594 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 594 dye is pH-insensitive over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

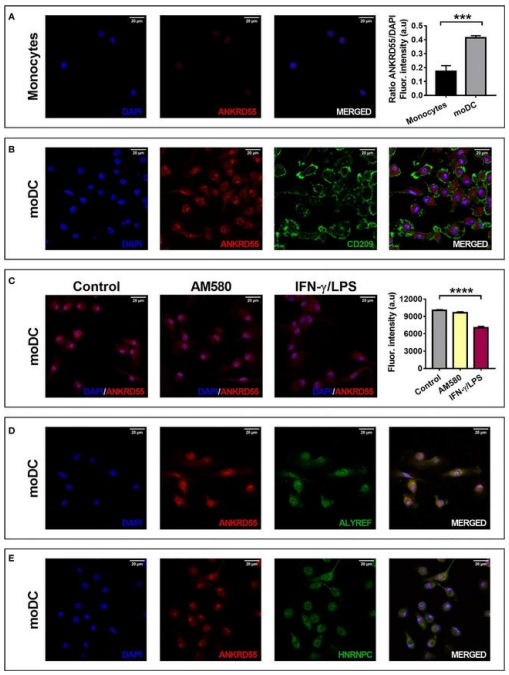
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

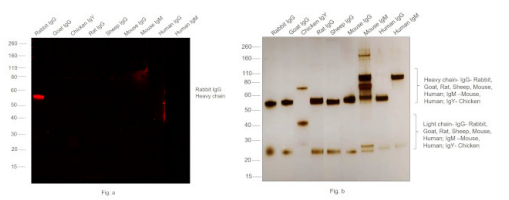
Product Images For F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11072) in WB
Multiplexed fluorescent western blot was performed using F(ab)2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Product # A-11072). Whole cell extracts of SH-SY5Y (Lane 1, 2, 3, 4), HeLa (Lane 5, 6, 7, 8), MCF7 (Lane 9), A-431 (Lane 10), HaCaT (Lane 11) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23002) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with Vimentin Polyclonal Antibody (Product # PA5-27231) and GAPDH Loading Control Monoclonal Antibody (GA1R) (Product # MA5-15738). Secondary antibodies (Product # A-11072, 1:5000 and Product # A32789, 1:20,000) were used for detection of Vimentin and GAPDH respectively, using the iBright™ FL 1500 (Product # A44115). The F(ab)2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Product # A-11072) specifically detects the Vimentin rabbit primary antibody.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11072) in ICC/IF
Localization of ANKRD55 in (A) monocytes (1.024 × 1.024 pixels = 0.131 microns /pixel) and (B-E) moDC by immunofluorescence microscopy. Rightmost graph represents mean ± SEM in monocytes and immature moDC (n = 5 cellular ROIs /group; unpaired t-test). (B) Colocalization of ANKRD55 with CD209, a membrane marker specific for moDC (2.048 × 2.048 pixels = 0.065 microns /pixel). (C) Effect of AM580 and IFN-/LPS treatment on nuclear ANKRD55 signal in immunofluorescence (1,808 × 1,808 pixels = 0,074 microns/pixel). The diagram on the right provides quantitative analysis of nuclear ANKRD55 immunofluorescence [mean ± SEM; n 59 cellular ROIs/condition; Kruskal-Wallis test (followed by Dunn's multiple comparison test)]. (D) Colocalization of ANKRD55 with ALYREF (1.024 × 1.024 pixels = 0.131 microns/pixel). (E) Colocalization of ANKRD55 with HNRNPC (1.024 × 1.024 pixels = 0.131 microns /pixel). ***p 0.001, ****p 0.0001. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35111166), licensed under a CC BY license.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11072)
Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG. A band at ~55 kDa corresponding to Rabbit IgG Heavy Chain was observed in Rabbit IgG but not in other species using F(ab)2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Product # A-11072) in Western Blot. {RE}

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272 References

Dimerization of the 4Ig isoform of B7-H3 in tumor cells mediates enhanced proliferation and tumorigenic signaling. Commun Biol (2024)

In vitro production of cat-restricted Toxoplasma pre-sexual stages. Nature (2024)

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The FAM104 proteins VCF1/2 promote the nuclear localization of p97/VCP. Elife (2023)

Moderate Intensity of Treadmill Exercise Rescues TBI-Induced Ferroptosis, Neurodegeneration, and Cognitive Impairments via Suppressing STING Pathway. Mol Neurobiol (2023)