

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

Product Details	
Size	1 mg
Species Reactivity	Human
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_2535745

Applications	Tested Dilution	Publications
Western Blot (WB)	1:2,000	-
Immunohistochemistry (IHC)	1-10 µg/mL	-
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information
Product will be shipped at Room Temperature.

Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21089)

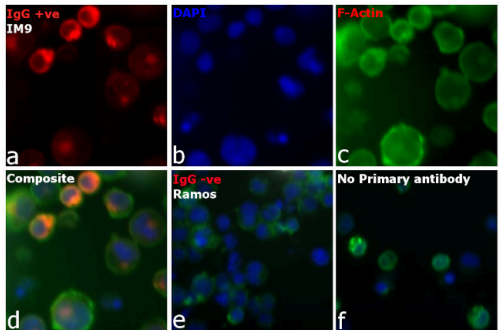
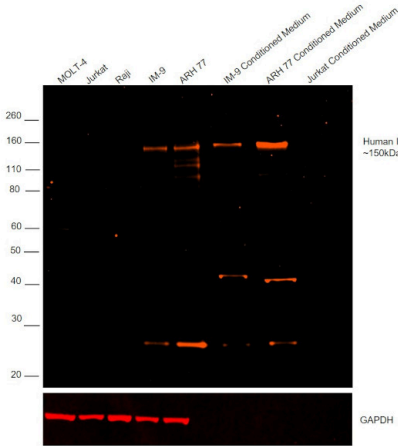
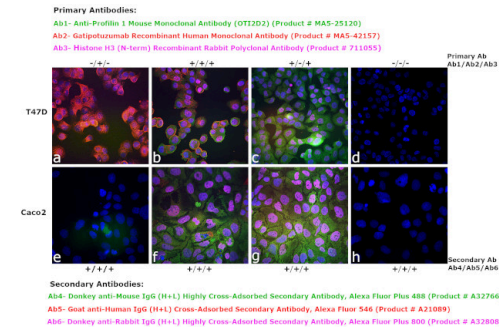
Immunofluorescence analysis of Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-21089) was performed using anti-Human MUC1 antibody (Product # MA5- 42157) with T-47D (positive) and Caco-2 (negative) cells. The cells were fixed with 4% Paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 2% BSA, then incubated with following primary antibodies at 1:100 dilution at 4 degree celsius overnight; Panel a & e- Ab2 (MUC1), Panel b & f- Ab1 (PFN1), Ab2 (MUC1), Ab3 (H3) and Panel c & g-: Ab1 (PFN1), Ab3 (H3). The cells were then incubated with secondary antibodies- Ab4 (Product # A32766), Ab5 (Product # A-21089) and Ab6 (Product # A32808) in 0.1% BSA at room temperature for 45 minutes. The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR) and externally deconvoluted (D.Sage et al./Methods 115 (2017) 28–41). Panel a shows plasma membrane localization of MUC1 in T-47D and its absence in Caco-2 (panel e), Panel b shows plasma membrane, cytoplasmic and nuclear localization of MUC1, PFN1 and H3 respectively in the channels corresponding to the wavelength of their secondary antibody. Panel c shows no signal in red channel proving the specificity of Ab4 to human primary antibody and not to mouse or rabbit primary antibody. Panel f & g show Caco-2 with PFN1 and H3 staining. Panel d & h are control cells with no primary antibody. {RE}

Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21089)

Antibody specificity was demonstrated by detection of differential basal expression of IgG across cell lines owing to their inherent genetic constitution. Relative expression of Human IgG was observed in IM-9, ARH-77 and IM-9, ARH-77 conditioned medium (CM) but not in Raji, MOLT-4, Jurkat and Jurkat CM using Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-21089) in Western Blot. IM-9 and ARH-77 express and secrete IgG whereas Raji is known to express IgM. MOLT-4 and Jurkat (T-cell lines) do not express immunoglobulins. (DOI:10.1002/eji.1830100305;10.3791/3573;10.1016/0022-1759(94)00286-6;PMID: 566614). {RE}

Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21089) in ICC/IF

Immunofluorescence analysis of Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 was performed using log phase IM9 cells (IgG producing B-cell line). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-21089) at 1:250 dilution in 0.1% BSA, incubated at 4 degree celsius overnight (Panel a: red). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin (Panel c: green) was stained with Alexa Fluor™ 488 Phalloidin (Product # A12379, 1:300 dilution). Panel d represents the merged image showing cytoplasmic (plasma membrane and golgi-body like) localization. Panel e represents Ramos (IgG non-producing B-cell line) which is a negative model for IgG expression. Panel f represents control cells with isotype control antibody to assess background. The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR) and externally deconvoluted (D.Sage et al. /Methods 115 (2017) 28–41).



31 References

Structural basis for antibody-mediated NMDA receptor clustering and endocytosis in autoimmune encephalitis. *Nat Struct Mol Biol* (2024)

IKK inhibits cognitive memory and adult hippocampal neurogenesis via the β -catenin pathway *Research Square* (2024)

The central helicase domain holds the major conformational epitopes of melanoma differentiation-associated gene 5 autoantibodies. *Rheumatology (Oxford)* (2024)

Electrostatic properties of human germlines and biodistribution of small biologics. *MAbs* (2024)

Spindle assembly checkpoint insensitivity allows meiosis-II despite chromosomal defects in aged eggs. *EMBO Rep* (2023)

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