Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647

Product Details	
Size	1 mg
Species Reactivity	Human
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 647
Excitation/Emission Max	650/671 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_2535862

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

Product Specific Information

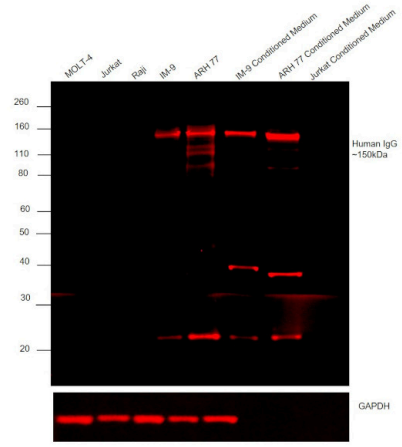
To minimize cross-reactivity, these goat anti-human IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against mouse, rabbit, and bovine serum prior to conjugation. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting 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 647 dye is a near-infrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 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 647 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.

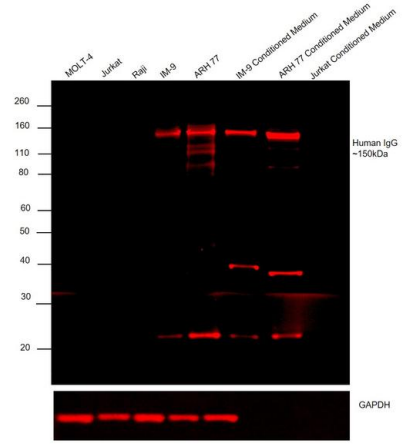
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

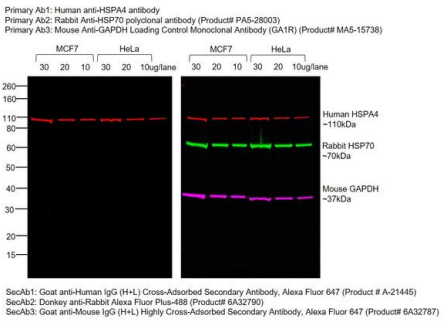
Product Images For Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647



Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21445)
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 647 (Product # A-21445) 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-21445) in WB
Western blot (non-reducing) was performed using Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Product # A-21445) and a 150 kDa band corresponding to Human IgG was observed in IM-9, ARH-77 and IM-9, ARH-77 conditioned medium8203 (CM) but not in Raji, Jurkat, Molt-4 and Jurkat CM which are known to have low expression. Whole cell lysate (30 µg) of MOLT-4 (Lane 1), Jurkat (Lane 2), Raji (Lane 3), IM-9 (Lane 4), ARH-77 (Lane 5), IM-9 CM (Lane 6), ARH-77 CM (Lane 7) and Jurkat CM (Lane 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Product # A-21445)(1:2000 dilution) and detected by fluorescence using iBright FL1500 (Product # A44115). 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).



Human IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21445) in WB
Multiplexed western blot analysis was performed using Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Product # A-21445). Whole cell extract of MCF7 (30, 20, 10 µg) (Lane 1, 2, 3) and HeLa (Lane 4, 5, 6) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with primary Ab1, Ab2, Ab3 (0.5 µg /mL) at 4 degree Celsius overnight. The blot was then probed with SecAb1, SecAb2 and SecAb3 (1:10000 dilution) and detected by fluorescence using the iBright FL1500 (Product # A44115). Specificity of Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Product # A-21445) towards Human IgG was demonstrated by the specific detection of band corresponding to HSPA4 (~110 kDa) (Fig a). Absence of detection of Rabbit HSP70 (~70 kDa) and Mouse GAPDH (~37 kDa) in the Alexa Fluor 647 channel confirms specificity. Fig b shows multiplexed detection of all three fluorescently labeled secondary antibodies. Sensitivity of Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Product # A-21445) is demonstrated by detection of HSPA4 (~110 kDa) across all three lysate concentrations (30, 20, 10 µg/lane).

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371 References

Identification and validation of a novel autoantibody biomarker panel for differential diagnosis of pancreatic ductal adenocarcinoma. Front Immunol (2025)

High-throughput multiplexed serology via the mass-spectrometric analysis of isotopically barcoded beads. Nat Biomed Eng (2025)

Enzymatic conversion of blood group B kidney prevents hyperacute antibody-mediated injuries in ABO-incompatible transplantation. Nat Commun (2025)

A truncated pre-F protein mRNA vaccine elicits an enhanced immune response and protection against respiratory syncytial virus. Nat Commun (2025)

The single amino acid change of R516K enables efficient generation of vesicular stomatitis virus-based Crimean-Congo hemorrhagic fever reporter virus bioRxiv (2025)