Goat anti-Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568

Product Details	
Size	500 µg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 568
Excitation/Emission Max	579/603 nm
Immunogen	Gamma Immunoglobin Fc region
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, DO NOT FREEZE!
RRID	AB_2925778

Applications	Tested Dilution	Publications
Western Blot (WB)	1 µg/mL	-
Immunohistochemistry (IHC)	Assay-dependent	-
Immunocytochemistry (ICC/IF)	1-2 µg/mL	-
Flow Cytometry (Flow)	2-8 µg/mL	-

Product Specific Information

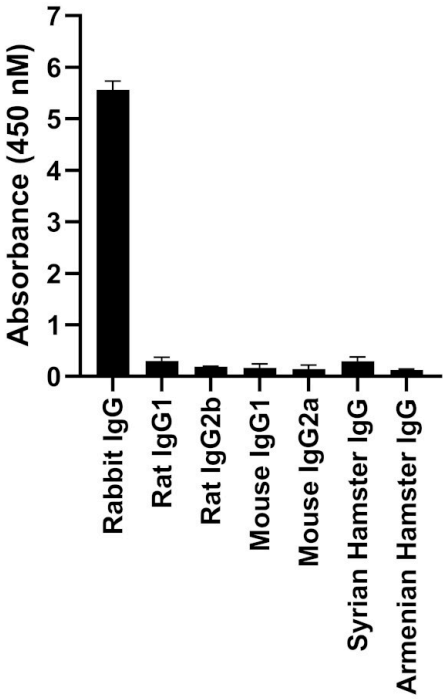
This goat anti-rabbit IgG Fc secondary antibody has been affinity-purified and shows minimum cross-reactivity to human, mouse and rat serum proteins. These antibodies have been solid phase adsorbed to ensure class specificity and cross adsorbed using human, mouse and rat immunosorbents to remove cross reactive antibodies. 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 secondary antibodies 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 may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright, orange/red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 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 568 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.

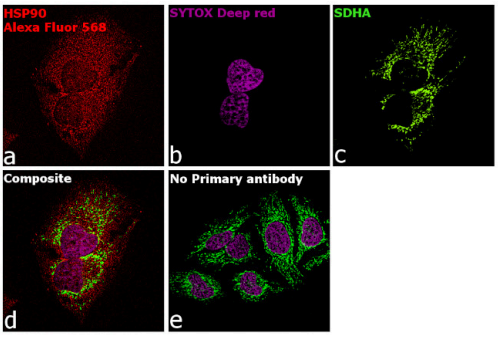
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because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

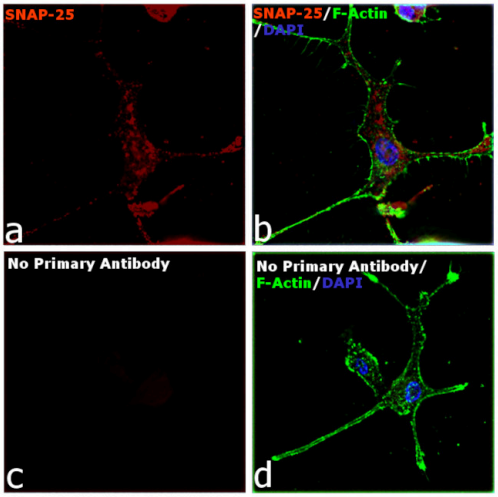
Product Images For Goat anti-Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568



Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody (A78955)
Antibody specificity of Goat anti-Rabbit IgG Fc cross adsorbed antibody was demonstrated in ELISA by quantifying detection of the specific immunoglobulin of interest along with closely related immunoglobulins. The assay was performed by coating different immunoglobulins at 100 ng/well. The coated proteins were incubated with unconjugated Goat anti-Rabbit IgG Fc cross adsorbed antibody (1: 4,000 dilution) and detected using Chicken anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP (Product # A15969, 1:5,000 dilution). The plate was developed using TMB stabilized chromogen solution (Product # SB02). The plate was read at 450 nm with Thermo Scientific™ Varioskan™ LUX multimode microplate reader (Product # VLBLATD2). The antibody demonstrates specificity towards Rabbit IgG with no cross-reactivity towards various species immunoglobulins as specified in the legend, including mouse and rat IgG. {ARRAY}



Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody (A78955) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG Fc, Cross Adsorbed Secondary Antibody, Alexa Fluor™ 568 (Product # A78955, 1:2,000 dilution) was performed on fixed and permeabilized HeLa cells stained with HSP90 alpha Polyclonal Antibody (Product # PA3-013, 1:2000 dilution). Panel a) shows representative cells that were stained for detection and localization of HSP90 protein, Panel b) is stained for nuclei using ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red (Product # P36990). Panel c) represents mitochondrial staining using Mouse SDHA antibody and appropriate secondary antibody conjugated to Alexa Fluor™ 488. Panel d) is a composite image of Panels a, b and c clearly demonstrating specific detection of Rabbit HSP90 antibody in the cytoplasm using Goat anti-Rabbit IgG Fc, Cross Adsorbed Secondary Antibody, Alexa Fluor™ 568. Panel e) represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody (A78955) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG Fc, Cross Adsorbed Secondary Antibody, Alexa Fluor™ 568 (Product # A78955) at a 1:2,000 dilution was performed on fixed and permeabilized PC-12 cells that were subjected to neuronal differentiation. The cells were stained with Anti-SNAP25 Recombinant Rabbit Monoclonal Antibody (Product # 701991) at a 1:100 dilution. Panel a) shows representative images of cells that were stained for detection and localization of SNAP25 protein, Panel b) is a composite of panel a, the nuclei stained using ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962) and cytoskeletal F-actin staining stained with Alexa Fluor™ 488 Phalloidin (Product # 12379) at a 1:300 dilution. Panel c) shows representative images of cells with no primary antibody to assess the background. Panel d) is a composite of panel c, the nuclei, and F-actin. The images were captured at 60X magnification.

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