Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680

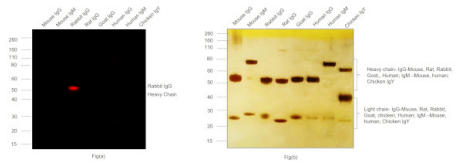
Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 680
Excitation/Emission Max	687/704 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	proprietary buffer, pH 6.5
Contains	0.016% Bromonitrodioxane, 0.016% Methylisothiazolone
Storage conditions	4° C, store in dark
RRID	AB_2633283

Applications	Tested Dilution	Publications
Western Blot (WB)	0.02-0.1 µg/mL	0 Publication
Immunocytochemistry (ICC/IF)	1:2000	-
Flow Cytometry (Flow)	1:50	-
Miscellaneous PubMed (Misc)	-	0 Publication

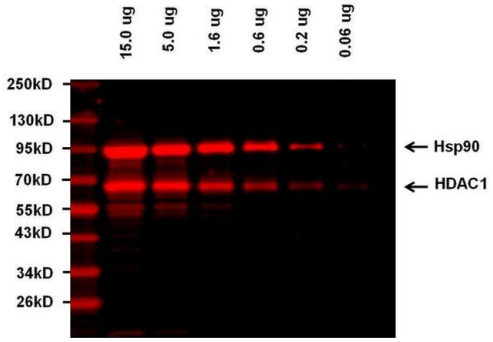
Product Specific Information

To minimize cross-reactivity, the goat anti-rabbit IgG whole antibodies have been pre cross-adsorbed against bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in less background staining and cross-reactivity. 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. Further passages through additional columns result in highly cross-adsorbed preparations of secondary antibody. The benefits of these extra steps are apparent in multiplexing/multicolor-staining experiments 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.^M
^M
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.

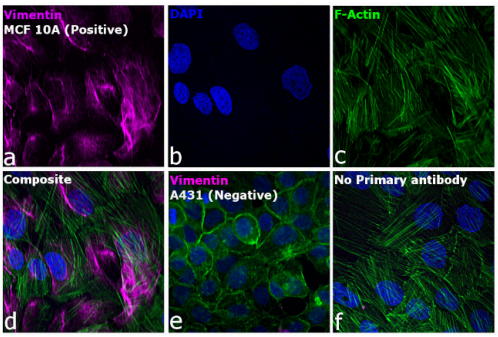
Product Images For Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32734)
Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG (H+L). A band at ~50 kDa and 25 kDa Heavy and Light Chain was observed in Rabbit IgG but not in other species using Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32734) in Western Blot.Relative expression. {RE}



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32734) in WB
Western blot analysis of Heat Shock Protein 90 (Hsp90) and histone deacetylase 1 (HDAC1) was performed by loading 3-fold serial dilutions of A431 whole cell lysate (starting at 15 µg) and 2 µL of the PageRuler Prestained NIR Protein Ladder (Product # 26635) per well onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to Nitrocellulose Membranes (Product # 88018) and blocked with Fluorescence Blocker for 30 min. Membranes were probed with a Hsp90 polyclonal antibody (Product # PA3-013) at a dilution of 1:5000 and an HDAC1 polyclonal antibody at a dilution of 1:5000 (Product # PA1-1110) overnight at 4°C on a rocking platform, washed with TBST, and probed with an Invitrogen Alexa Fluor Plus 680 Goat anti-Rabbit IgG secondary antibody (Product # A32734) at dilutions of 1:40,000 for 45 minutes. Blots were imaged on an Infrared fluorescence imaging system.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32734) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 680 (Product # A32734) was performed using MCF 10A (positive model) and A431 (negative model) cells stained with Vimentin Monoclonal Antibody (Product # MA1-19168). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/mL primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A32734, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Vimentin in the cytoskeleton (Panel a: pink). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:300) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in A431 (negative model for Vimentin) 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) and externally deconvoluted (D.Sage et al./Methods 115 (2017) 28–41).

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56 References

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