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

Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Donkey / 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% Methylisothiazolone, 0.016% Bromonitrodioxane
Storage conditions	4° C, store in dark
RRID	AB_2762841

Applications	Tested Dilution	Publications
Western Blot (WB)	0.1-0.4 µg/mL	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-

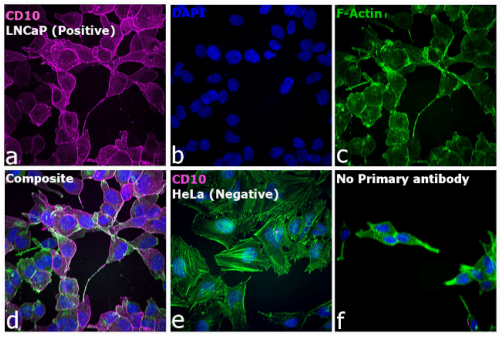
Product Specific Information

To minimize cross-reactivity, the donkey anti-goat IgG whole antibodies have been cross-adsorbed against IgG from human, mouse, rabbit, and rat, as well as non-immunoglobulin goat serum. 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.

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.

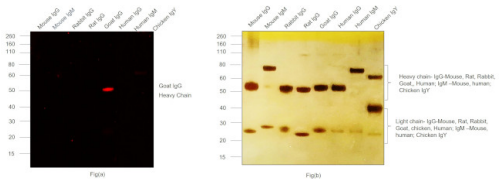
Specificity: This antibody binds to heavy chains on goat IgG and light chains on all goat immunoglobulins. This antibody does not bind non-immunoglobulin goat serum proteins or IgG from human, mouse, rabbit or rat.

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



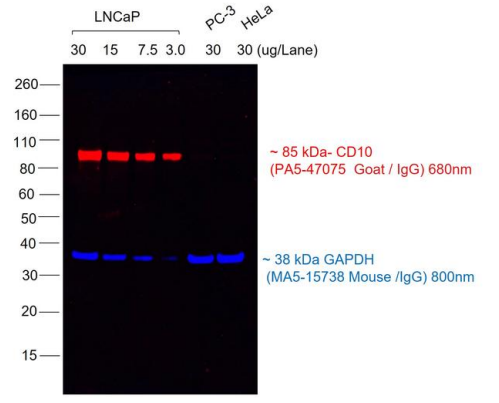
Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32860) in ICC /IF

Immunofluorescence analysis of Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32860) was performed using LNCaP (positive model) and HeLa (negative model) cells stained with CD10 Polyclonal Antibody (Product # PA5-47075). 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. Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32860, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of CD10 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 HeLa (negative model for CD10) 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).



Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32860)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Goat IgG. A band at ~50 kDa corresponding to Goat IgG Heavy Chain was observed in Goat IgG but not in other species using Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32860) in Western Blot.Relative expression. {RE}



Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32860) in WB

Multiplexed fluorescent western blot was performed using Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32860). Whole cell extracts of LNCaP (Lane 1, 2, 3, 4), PC-3 (Lane 5) and HeLa (Lane 6) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with CD10 Polyclonal Antibody (Product # PA5-47075) and GAPDH Loading Control Monoclonal Antibody (GA1R) (Product # MA5-15738). Secondary antibodies (Product # A32860, 1: 10000 dilution) and (Product # A32789, 1:20000 dilution) were used for detection of CD10 and GAPDH respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-goat secondary antibody (Product # A32860) specifically detects the goat primary antibody.

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7 References

Smooth muscle protein 22-Cre recombination in resting cardiac fibroblasts and hematopoietic precursors. Sci Rep (2022)

An ex vivo system to study cellular dynamics underlying mouse peri-implantation development. Dev Cell (2022)

Kainate receptors regulate the functional properties of young adult-born dentate granule cells. Cell Rep (2021)

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Fc receptor IIIa/CD16a processing correlates with the expression of glycan-related genes in human natural killer cells. J Biol Chem (2020)