Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488

Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 488
Excitation/Emission Max	493/518 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_2762838

Applications	Tested Dilution	Publications
Western Blot (WB)	0.1-0.4 µg/mL	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

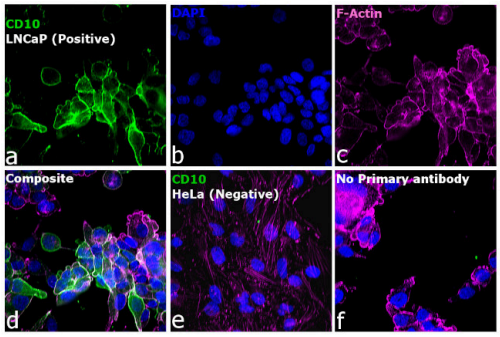
Product Specific Information

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.

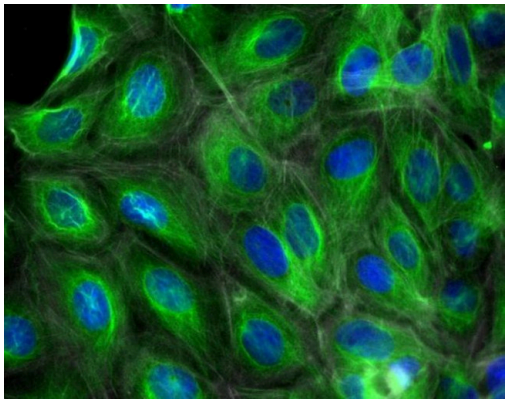
Specificity: This antibody binds to heavy chains on goat IgG and light chains on all goat immunoglobulins. This antibody does not bind non-immunoglobulin goat serum proteins or IgG from human, mouse, rabbit or rat.

Product Images For Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488



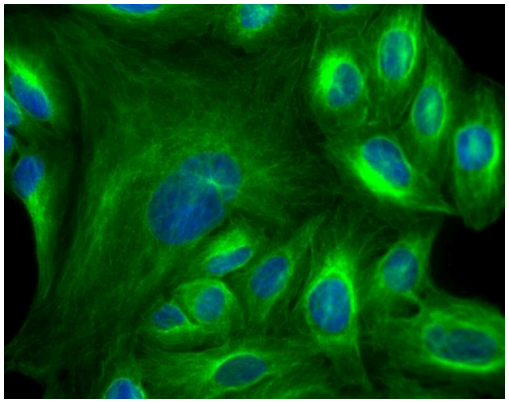
Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32814) in ICC /IF

Immunofluorescence analysis of Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32814) 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 488 (Product # A32814, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Vimentin in the cytoplasm (Panel a: green). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor™ 647 Phalloidin (Product # A22287, 1:4000) (Panel c: pink). 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). Nonspecific 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 (A32814) in ICC /IF

Immunofluorescent analysis of tubulin in U2OS cells. The cells were fixed with 4% formaldehyde for 20 mins, permeabilized with 0.5% Triton X-100 in PBS for 20 mins, washed 3X in PBS and blocked with 3% BSA in PBS for 30 mins at RT. Cells were stained with a tubulin antibody at a dilution of 1:500 in 3% BSA in PBS over night at 4C, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 488 donkey anti-goat IgG secondary antibody (Product # A32814) prepared in 3% BSA in PBS at a dilution of 1:500 for 1 hr at RT in the presence of NucBlue Live ReadyProbes Reagent (Product # R37605). The image contains overlay of tubulin (green) and nuclei (blue). Images were taken on an EVOS FL Auto 2 Imaging System (Product # AMAFD2000) with an Olympus 40X Super Apochromat objective (Product # AMEP4754) at 40X magnification. Actin was stained using Alexa Fluor Plus Phalloidin (Product # A30105).



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202 References

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