Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 555

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 555
Excitation/Emission Max	558/572 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_2762848

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

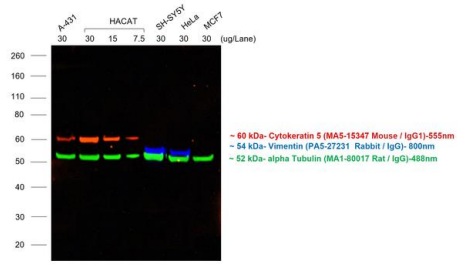
Product Specific Information

To minimize cross-reactivity, the donkey anti-mouse IgG whole antibodies have been cross-adsorbed against IgG from bovine, goat, chicken, guinea pig, hamster, horse, sheep, rabbit, rat, and human. 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.

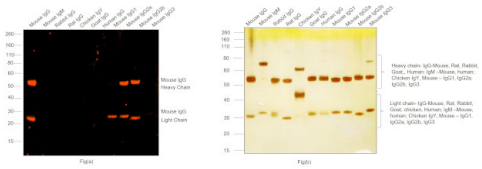
Specificity: This antibody binds to heavy chains on mouse IgG and light chains on all mouse immunoglobulins. This antibody does not bind non-immunoglobulin mouse serum proteins or IgG from bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat or sheep.

Product Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 555



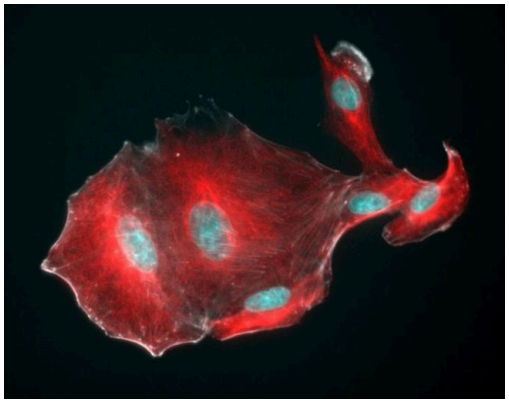
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32773) in WB

Multiplexed fluorescent western blot was performed using Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555 (Product # A32773). Whole cell extracts of A-431 (Lane 1), HaCaT (Lane 2, 3, 4), SH-SY5Y (Lane 5), HeLa (Lane 6) and MCF7 (Lane 7) 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 Cytokeratin 5 Monoclonal Antibody (3E2F1) (Product # MA5-15347), Vimentin rabbit IgG Polyclonal Antibody (Product # PA5-27231) and alpha Tubulin Monoclonal Antibody (YL1/2) (Product # MA1-80017). Secondary antibodies (Product # A32773, 1:10000 dilution), (Product # A32734, 1:20000 dilution) and (Product # A48269, 1:10000 dilution) were used for detection of Cytokeratin 5, Vimentin and alpha Tubulin respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # A32773) specifically detects the mouse primary antibody and not the rabbit and the rat primary antibodies.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32773)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Mouse IgG. A band at ~50 and 25 kDa corresponding to Mouse IgG Heavy and Light Chain was observed in Mouse IgG, IgG1, IgG2a, IgG2b but not in other species using Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555 (Product # A32773) in Western Blot. {RE}



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32773) in ICC/IF

Immunofluorescent analysis of tubulin in A549 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 1:2000 in 3% BSA in PBS for 1 hr at RT, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 555 donkey anti-mouse IgG secondary antibody (Product # A32773) prepared in 3% BSA in PBS at a dilution of 1:1000 for 1 hr at RT in the presence of NucBlue Live ReadyProbes Reagent (Product # R37605). The image contains overlay of tubulin (red) 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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85 References

Regulation of the apico-basolateral trafficking polarity of the homologous copper-ATPases ATP7A and ATP7B. J Cell Sci (2024)

HAMSAB diet ameliorates dysfunctional signaling in pancreatic islets in autoimmune diabetes. iScience (2024)

Multiplexed CRISPR gene editing in primary human islet cells with Cas9 ribonucleoprotein. iScience (2024)

Potential mechanism of TMEM2/CD44 in endoplasmic reticulum stressinduced neuronal apoptosis in a rat model of traumatic brain injury. Int J Mol Med (2023)

Preadipocytes in human granulation tissue: role in wound healing and response to macrophage polarization. Inflamm Regen (2023)