Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 800

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 800
Excitation/Emission Max	789/794 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_2762832

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

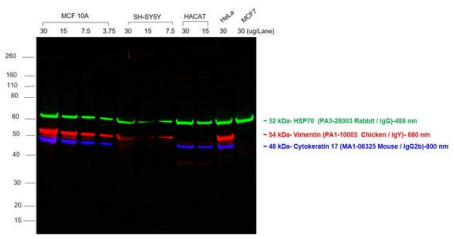
Product Specific Information

To minimize cross-reactivity, the donkey anti-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

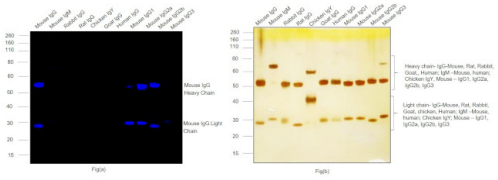
Specificity: This antibody binds to heavy chains on mouse IgG and light chains on all mouse immunoglobulins. This antibody does not bind non-immunoglobulin mouse serum proteins or IgG from bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat or sheep.

Product Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 800



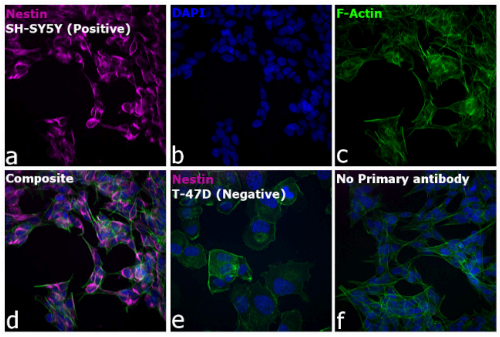
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32789) in WB

Multiplexed fluorescent western blot was performed using Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 800 (Product # A32789). Whole cell extracts of MCF 10A (Lane 1, 2, 3, 4), SH-SY5Y (Lane 5, 6, 7), HaCaT (Lane 8, 9), HeLa (Lane 10) and MCF7 (Lane 11) 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 Vimentin Chicken IgY Polyclonal Antibody (Product # PA1-10003), Cytokeratin 17 mouse IgG2b Monoclonal Antibody (E3) (Product # MA1-06325) and HSP70 rabbit Polyclonal Antibody (YL1/2) (Product # PA5-28003). Secondary antibodies (Product # A32934, 1:20000), (Product # A32789, 1:20000) and (Product # A32790, 1:20000) were used for detection of Vimentin, Cytokeratin 17 and alpha Tubulin respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # A32789) specifically detects the mouse primary antibody and not the rabbit and the rat primary antibodies.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32789)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Mouse IgG (H+L). A band at ~50 kDa and 25 kDa Heavy and Light Chain was observed in Mouse IgG, Mouse IgG1, Mouse IgG2a, Mouse IgG2b and Mouse IgG3 but not in other species using Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 800 (Product # A32789) in Western Blot.Relative expression. {RE}



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32789) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 800 (Product # A32789) was performed using SH-SY5Y (positive model) and T-47D (negative model) cells stained with Nestin Monoclonal Antibody (Product # 14-9843-80). 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-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 800 (Product # A32789, 1:2000) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Nestin in the cytoplasm (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 T-47D(negative model for vimentin) 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).

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11 References

Cholinergic neurodegeneration and cholesterol metabolism dysregulation by constitutive p75NTR signaling in the p75exonIII-KO mice. Front Mol Neurosci (2023)

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