

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

Product Details		
Size	100 μg	
Species Reactivity	Mouse	
Host/Isotype	Donkey / IgG	
Class	Polyclonal	
Туре	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_2866493	

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

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.^M

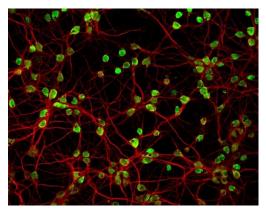
^M

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^M

^M

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 488

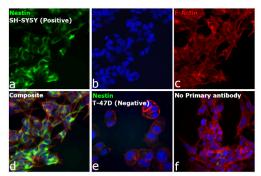


Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32766TR) in ICC/IF

Immunofluorescent analysis of HuC/D and MAP-2 in Rat cortical neurons. Gibco Rat Cortex Neurons (Product # A1084001) were thawed and grown according to protocol using B-27 Plus Neurobasal Culture System (Product # A3653401) and GlutaMAX (Product # 35050061) for two weeks before processing with the Image-IT Fixation/Permeablization kit (Product # R37602) according to protocol. Cells were blocked with 3% BSA in PBS for 30 mins at RT, incubated with a HuC/D mouse monoclonal antibody (Product # A21271) and a rabbit anti MAP-2 antibody (Product # PA517646) at a dilution of 1:500 in 3% BSA in PBS for 1 hr at RT, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 488 donkey anti-mouse IgG secondary antibody (Product # A32766) and Invitrogen Alexa Fluor Plus 594 donkey anti-rabbit IgG secondary antibody (Product # A32754) prepared in 3% BSA in PBS at a dilution of 1:1000 for 1 hr at RT. The image contains overlay of HuC/D (green) and MAP-2 (red). Images were taken on an EVOS FL Auto 2 Imaging System (Product # AMAFD2000) with an Olympus 20X Super Apochromat objective (Product # AMEP4734) at 20X magnification.

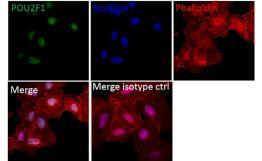
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32766TR) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32766) was performed using SH-SY5Y (positive model) and T-47D (negative model) cells stained with Nestin Monoclonal Antibody (10C2), eBioscience™ (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 488 (Product # A32766, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Nestin in the cytoskeleton (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: red). 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 Nestin) 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).



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32766TR) in ICC/IF

Immunofluorescent analysis of POU2F1 (green) in A549 cells (60 % confluency). The cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 for 10 minutes at 37 C, and blocked with 10% Normal Donkey Serum in PBS for 1 hour at room temperature. Cells were then stained with OCT1 (POU2F1) Monoclonal Antibody, Unconjugated at 1.25 µg/mL Mouse IgG1 isotype control at room temperature in blocking buffer, followed by Donkey anti-Mouse IgG (H+L) Alexa Fluor Plus 488 (Product # A32766) at 1:5000 in blocking buffer for 1 hour at room temperature. Cell nuclei were stained with NucBlueTM stain in ProLong™ Glass Antifade Mount (Product # P36985) (blue). The cytoskeleton was visualized using Rhodamine Phalloidin (red) (Product # R415). Images were acquired at 40x magnification with a Zeiss LSM 800 confocal. The upper panel shows cells stained with clone YL15. No staining was observed with mouse IgG1 isotype control (lower panel, Merged isotype).



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□ 3 References

"Imaging Translation in Early Embryo Development" bioRxiv (2024)

Perisomatic Synaptic Targeting by Cholecystokinin-Basket Interneurons through NrCAM and Ankyrin B bioRxiv (2024)

An Endosomal Escape Trojan Horse Platform to Improve Cytosolic Delivery of Nucleic Acids. ACS Nano (2024)

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