Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 568
Excitation/Emission Max	579/603 nm
Immunogen	Gamma Immunoglobin
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2534013

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

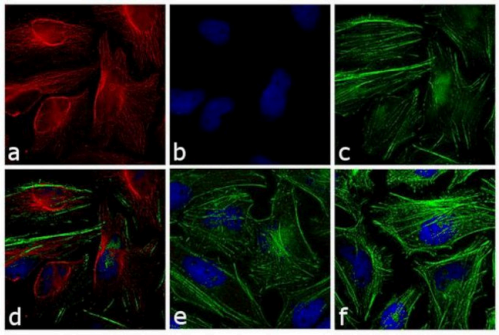
These donkey anti-mouse IgG whole secondary antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep serum proteins. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. 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. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) 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.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright, orange/red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 568 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

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. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

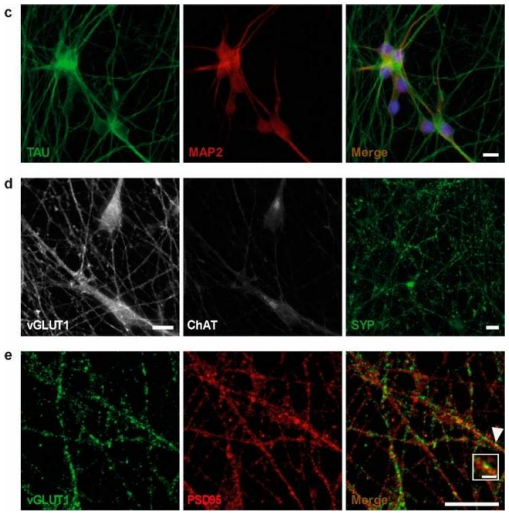
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568



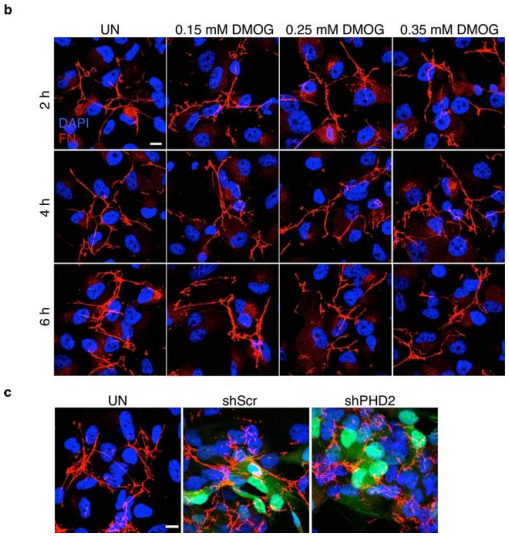
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10037) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 568 conjugate was performed using HeLa cells stained with alpha Tubulin (23610501) Mouse Monoclonal Primary Antibody (Product # A11126). 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 mouse primary antibody for 3 hours at room temperature. Donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 568 conjugate (Product # A10037) was used at a concentration of 2 µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10037) in ICC/IF

Differentiation and characterization of human induced pluripotent stem cell (iPSC) -derived cortical neurons. (a) Schematic representation of human iPSC differentiation protocol using doxycycline and coculturing of primary murine astrocytes. (b) Representative phase-contrast images at indicated time points of human iPSC differentiation into cortical neurons with and without coculturing of astrocytes; scale bar = 100 µm. (c-e) Representative images of iPSC-derived neurons 14 days after initiation of differentiation indicate proper neuronal maturation into glutamatergic neurons, and formation of axons, dendrites and synapses. (c) Immunocytochemical stainings of neuronal marker expression (TAU, MAP2) show the expected polarized distribution of predominantly axonal TAU (left panel) and predominantly dendritic MAP2 protein (middle panel). The merge includes nuclear stain NucBlue to indicate somata (right panel); scale bar = 10 µm. (d) Immunocytochemical stainings for neuronal differentiation markers. Neurons express glutamate transporter 1 (vGLUT1; left panel) but lack the expression of the motor neuron marker choline acetyltransferase (ChAT; middle panel). Neurons express the presynaptic marker synaptophysin (SYP; right panel); scale bars = 10 µm. (e) Immunocytochemical stainings for neuronal synaptic markers. Neurons form synapses, as visualized by expression and co-localization of the signals obtained with an… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov /33670788), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10037) in ICC/IF

DMOG treatment or PHD2 knockdown does not affect fibril assembly. (a) Caki-1 cells treated with different concentrations of DMOG at the indicated times were lysed and immunoblotted for HIF-1. -Actin was used as the loading control. (b) Cells treated as in (a) were immunostained for FN (red) and counterstained with the nuclear stain DAPI (blue). Scale bar = 10 µm. (c) Caki-1 cells transduced with shScr (200 MOI) or shPHD2 (2000 MOI) expressing the GFP reporter were immunostained for FN (red) and DAPI (blue). Transduced cells are shown in green (GFP reporter). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33122751), licensed under a CC BY license.

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926 References

Antisense oligonucleotide therapeutic approach for Timothy syndrome. Nature (2024)

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