Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 647
Excitation/Emission Max	650/671 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_162542

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
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
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these donkey anti-mouse IgG whole 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.

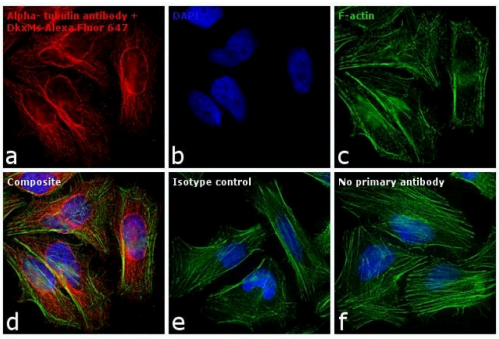
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 647 dye is a near-infrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 dye is pH-insensitive over 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 647 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.

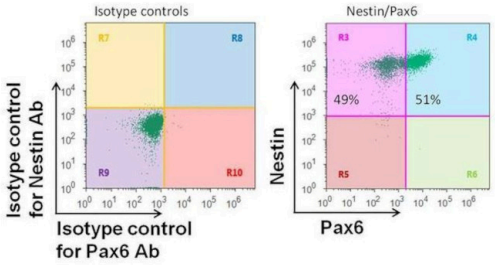
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647



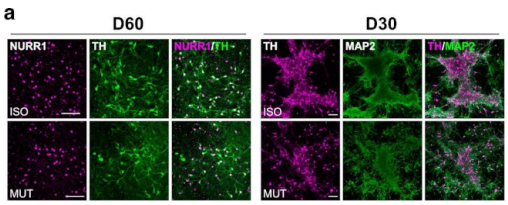
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31571) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 647 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal 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 primary antibody for 3 hours at room temperature. Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® 647 (Product # A-31571) 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 (A-31571) in Flow

Flow cytometry analysis of Pax6 on human neural stem cells derived from PD-3 iPSCs using Gibco® PSC Neural Induction Medium (Product # A1647801). Cells were fixed, permeabilized, and then stained with a Pax6 polyclonal antibody (Product # 42-6600) at a 1:100 dilution and a Nestin mouse monoclonal antibody (Product # MA1-110) at a 1:100 dilution. After incubation of the primary antibodies for 1 hour on ice, the cells were stained with Alexafluor® 488-conjugated goat anti-rabbit IgG secondary antibody (Product # A-11034) and Alexafluor® 647-conjugated donkey anti-mouse IgG secondary antibody (Product # A-31571) at a dilution of 1:500 for 1 hour on ice. Flow cytometry analysis was performed using the Attune® Acoustic Focusing Cytometer (Product # 4469120). A representative 10,000 cells were acquired for each sample.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31571) in ICC/IF

Secretome analysis on GBA1-PD-induced pluripotent stem cell (iPSC)-derived midbrain dopaminergic (mDA) neuron culture supernatant. (a) Representative immunostaining of mDA markers (NURR1, TH, and MAP2) on mDA neurons differentiated from GBA1 N409S mutant (MUT) or isogenic (ISO) iPSCs at day 60 (left) and 30 (right). Scale bars, 100 µm. (b) GCase activity assay on day 60 GBA1 N409S mutant and isogenic iPSC-derived mDA neurons (n = 4). (c) Volcano plot showing log2 fold change and log10 p-value in analyzed protein levels between GBA1 mutant and isogenic iPSC-derived mDA neuron culture supernatant (n = 6). (d) Forest plot displaying log2 fold change with SE of the proteins with a p-value < 0.05 in the supernatant analysis ordered in ascending p-values. Hit candidate iPSC proteins in common with the significantly altered CSF proteins are indicated in red. Hits found exclusively in the supernatant are indicated in black. Data are presented as the mean ± SEM. Two-tailed Student's t-test, * = p < 0.05. - Image collected and cropped by CiteAb under a CC-BY license from the following publication: Secretome Analyses Identify FKBP4 as a GBA1-Associated Protein in CSF and iPS Cells from Parkinson's Disease Patients with GBA1 Mutations. Int J Mol Sci (2024) Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/38203854), licensed under a CC BY license.

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2236 References

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Tac1-expressing neurons in the central amygdala predominantly mediate histamine-induced itch by receiving inputs from parabrachial Tac1-expressing neurons. Brain Res (2025)