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

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 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_2536180

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)	4 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.

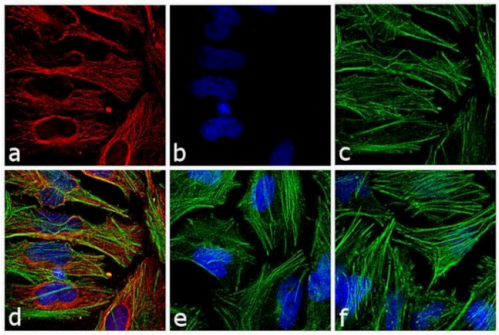
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive 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 555 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.

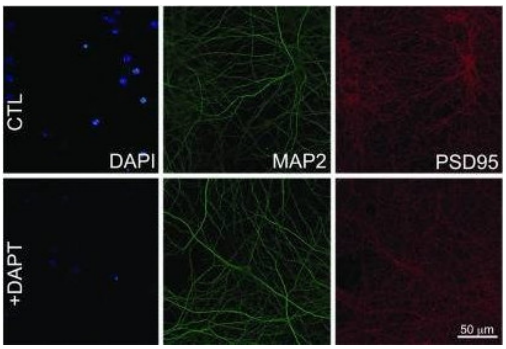
Product will be shipped at Room Temperature.

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



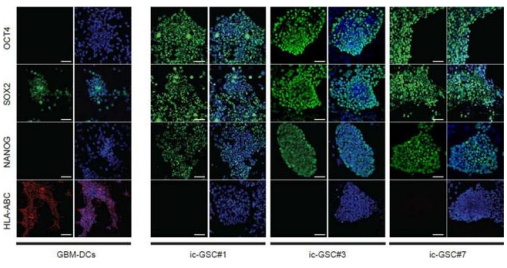
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31570) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 555 (Product # A-31570) 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 mouse primary antibody (1:250 dilution) for 3 hours at room temperature. Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 555 was used at concentration of 4µg/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: green). 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: red). 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-31570) in ICC/IF

T2DM reduces PSD95 levels in hAPP NL/F but not in WT mice.(A) Representative immunoblots of PSD95 in total lysates of brain cortical samples from control (CTL) or T2DM WT or hAPP-NL/F knockin (NL/F) mice. Actin was used as loading control. (A, B) The graph shows PSD95 protein levels (quantified from experiments as the one shown in panel (A)) normalized to the WT control group, (WT-CTL n = 9, WT-T2DM n = 10, NL/F-CTL n = 10, NL/F-T2DM n = 10). Statistical analysis was performed by one-way ANOVA (P = 0.0004), followed by Tukey's multiple comparisons test (*P < 0.05, ***P < 0.001). Unpaired t test also revealed a significant effect of T2DM in NL/F mice (#P = 0.0271, data normalized to NL/F control group). (C) DAPT treatment for 24 h in primary cortical neurons significantly decreased PSD95 protein levels determined by immunoblot (left). A plot comparing the average levels of PSD95 between control neurons (Ctl) and neurons treated with 10 µM DAPT (+DAPT), quantified from immunoblot experiments as the one shown in this panel, is shown on the right side of this panel (Ctl n = 6, DAPT n = 6). Statistical analysis was performed by paired t test (*P = 0.0461). (D) Representative confocal images for DAPI, MAP2, and PSD95 in control conditions (upper images) and treated with DAPT inhibitor (lower images). Scale bar: 50 µm. (D, E) The plot compa - Image collected and cropped by CiteAb under a CC-BY license from the fo… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov /37059474), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31570) in ICC/IF

Characterization of an in vitro induced core glioma stem cell model generated from glioblastoma patient-derived cells. (A) Workflow depicting the reprogramming of GBM-DCs into ic-GSC stable lines. (B) Immunostaining of OCT4, SOX2, NANOG, and HLA-ABC in GBM-DCs and three independent ic-GSC clones: ic-GSC#1, ic-GSC#3, and ic-GSC#7. Scale bars correspond to 50 µm. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35565200), licensed under a CC BY license.

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1641 References

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