Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 546
Excitation/Emission Max	561/572 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_2534012

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	-	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.

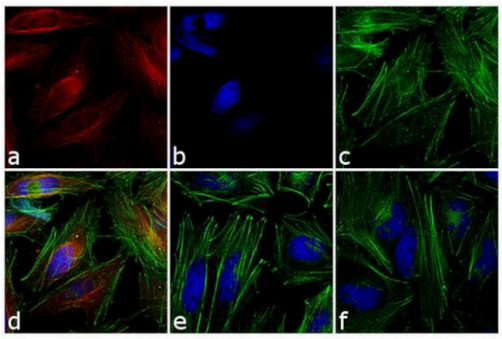
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 546 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 546 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 546 dye is pH-insensitive 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 546 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.

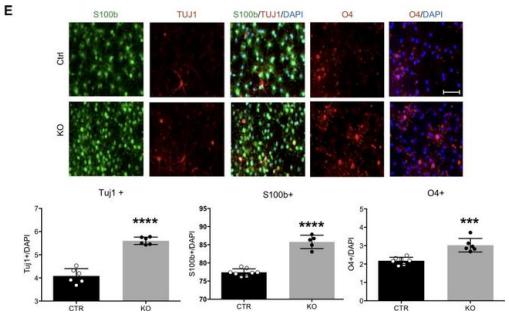
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546



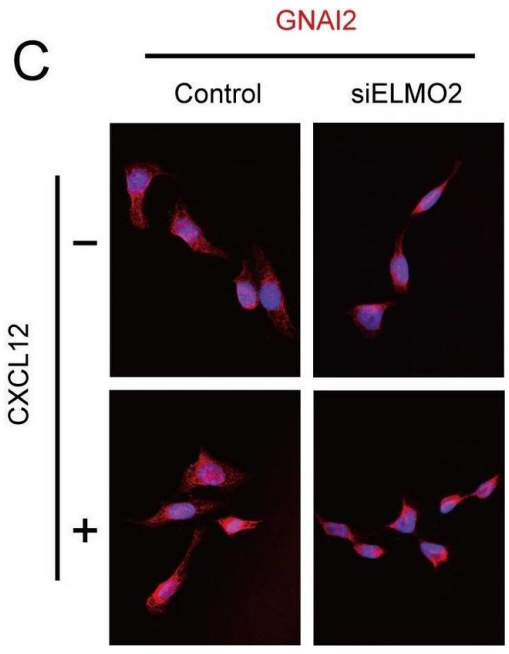
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10036) in ICC/IF

Immunofluorescence analysis of Donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 546 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 546 conjugate (Product # A10036) was used at a concentration of 4 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 (A10036) in ICC/IF

Loss of Tbl1xr1 affects neural stem cells dynamics. (A) Scheme of NSC derivation with examples of: genotyping (by PCR), expression of Tb1xr1 (mRNA abundance by RT-qPCR, ****p < 0.0001, unpaired t-test), and TBL1XR1 protein level (by Western blot). (B) NSCs both Ctrl and KO cultured as neurospheres, on the right quantification of spheres' diameter: difference due to genotype, F(1,40) = 63.19, ****p < 0.0001, 2-way ANOVA. (C) Growth curve of adherent in vitro NSCs: difference due to genotype, F(1,4) = 535.6, ****p < 0.0001, 2-way ANOVA. (D) Immunocytochemistry of both Ctrl and Tbl1xr1 KO proliferating NSCs for phosphor histone 3 (PH3) counterstained with DAPI. On the right, quantification of PH3+ cells on total DAPI nuclei, ****p < 0.0001, unpaired t-test. (E) Up, immunocytochemistry of both Ctrl and Tbl1xr1 KO differentiating NSCs for S100b (astrocytes), TUJ1 (neurons) and O4 (oligodendrocytes) counterstained with DAPI. Bottom, quantification: TUJ1: ****p < 0.0001, unpaired t-test; S100b: ****p < 0.0001, unpaired t-test; O4: ***p = 0.0002, unpaired t-test. Scale bars: (B) 40 µm; (D,E) 50 µm. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33708771), licensed under a CC BY license.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10036) in ICC/IF

CXCL12 stimulation results in ELMO2 membrane translocation.(A, B) Plasma membrane colocalizations of Gi2 and ELMO2 were evident upon pancreatic cancer cell stimulation with CXCL12. The extent of colocalization was calculated through ImageJ software. (C, D) No significant changes in plasma membrane-associated Gi2 fluorescence were detected in ELMO2 knockdown cells, even with CXCL12 stimulation. One-way ANOVA, p > 0.05. (E) Western blot clearly shows the Gi2 knockdown in siRNA-transfected PANC-1 cells. GAPDH was used as a loading control. (F, G) ELMO2 membrane translocation was reduced in Gi2 knockdown cells, even in the presence of CXCL12 stimulation. Twenty-five images were analyzed by ImageJ software. One-way ANOVA, p < 0.001. The arrows indicated the plasma membrane colocalization of Gi2 or ELMO2. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32292657), licensed under a CC BY license.

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418 References

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