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

Product Details	
Size	1 mg
Species Reactivity	Rat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 488
Excitation/Emission Max	499/520 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_2535794

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

Product Specific Information

These donkey anti-rat IgG (H+L)whole secondary antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rabbit, 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.

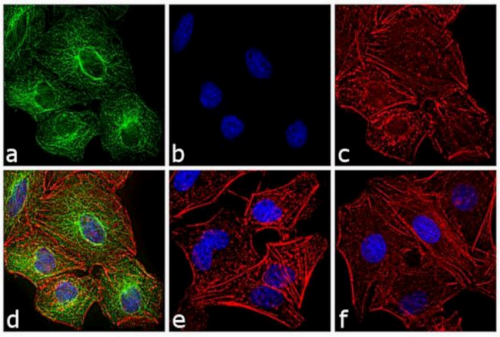
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over 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 488 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.

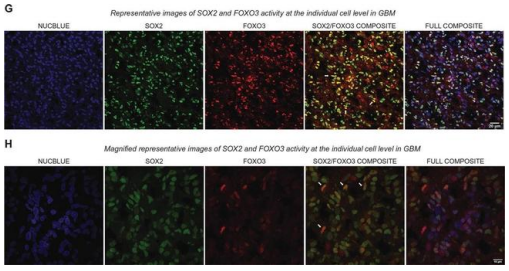
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



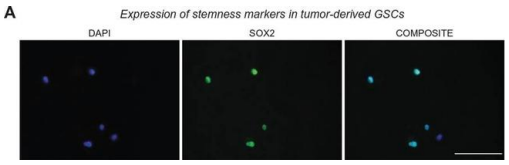
Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in ICC /IF

Immunofluorescence analysis of Donkey anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate was performed using A549 cells stained with alpha Tubulin (YL1/2) Rat Monoclonal Antibody (Product # MA1-80017). 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 2µg/mL Rat primary antibody for 3 hours at room temperature. Donkey anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate (Product # A-21208) was used at a concentration of 1µg/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 Rhodamine Phalloidin (Product # R415, 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.



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in ICC /IF

FOXO3 activity predicts SOX2 activity in GBM. (A) Immunohistochemistry of FOXO3 in a GBM tumor sample showing strongly positive (nuclear; active) FOXO3. (Scale bar 50 µm). (B) Immunohistochemistry of FOXO3 in a GBM tumor showing only weak cytoplasmic expression of FOXO3 in the cytoplasm of many tumor cells but not in the tumor cell nuclei or in adjacent endothelial cells. (C) Immunohistochemistry of stemness markers FOXO3 and SOX2, and the corresponding hematoxylin and eosin (H&E) staining in a GBM with high FOXO3 positivity (nuclear; active), and (D) immunohistochemistry of FOXO3 and SOX2 and the corresponding H&E staining in a GBM with low FOXO3 (negative). (E) Quantitation of SOX2 and FOXO3 protein expression by immunohistochemistry identifying FOXO3 activity as nuclear (active; positive) or cytoplasmic (inactive; negative) and strong SOX2 nuclear expression as positive or negative. In tumors with strong FOXO3 expression, SOX2 expression is also usually strongly expressed. (F) Relationship between the percentage of cells with nuclear FOXO3 and the percent of cells showing co-localization of SOX2 and FOXO3 within the same cell as indicated by dual-label immunofluorescence experiments as illustrated in (G). (G) Confocal immunofluorescence images showing FOXO3 (red) and SOX2 (green) co-localization at the individual cell level in GBM tumor samples. In the merged FOXO3/SOX2 images, tumor cells co-expressing FOXO3 and S… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36303712), licensed under a CC BY license.



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in ICC /IF

Overexpression and activation of endogenous FOXO3 reduces proliferation in primary human glioma stem cells (GSCs) in vitro. (A) Immunocytochemistry of stem/progenitor markers SOX2 (green) DAPI (blue) and (B) OLIG2 (green) and DAPI (blue) in the primary human GSC culture GSC-1. (Scale bar 100 µm). (C-D) Quantification of cell proliferation (EdU+/total DAPI) upon overexpression of GFP, wild-type FOXO3 (FOXO3-WT) or constitutively active FOXO3 (FOXO3-CA) in three independent GSC populations. One-way Analysis of Variance (ANOVA) with Dunnett's multiple comparison test at $p < 0.05$, GSC-1 ****$p < 0.0001$; GSC-2 ****$p < 0.0001$, **$p = 0.0035$; GSC-3 ****$p < 0.0001$. GSC-3-two biological replicates. (Scale bar 100 µm) (E) Quantification of cell proliferation (EdU+/total DAPI) following activation (induced nuclear localization) of endogenous FOXO3 in GSC-1 through growth factor deprivation (GF) and/or treatment with the PI3K-inhibitor LY294002 for 16 h. One-way ANOVA with Dunnett's multiple comparison test at $p < 0.05$, ****$p < 0.0001$. All above experiments were performed in three biological replicates per condition. (F) Quantification of cell proliferation (EdU+/total DAPI) following ablation of FOXO3 (FOXO3) or control (FOXO3-SC) in GSC-1 or GSC-2. Student's t-test, three replicates per cell line. $p < 0.05$ Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36303712), licensed under a CC BY license.

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1573 References

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