Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594

Product Details	
Size	1 mg
Species Reactivity	Rat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 594
Excitation/Emission Max	590/618 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_2535795

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	1 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication
Not applicable (N/A)	-	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.

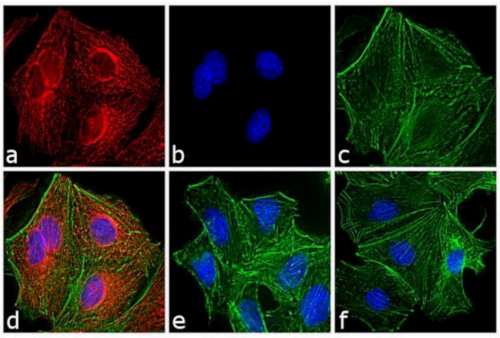
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 594 dye is a bright, red-fluorescent dye with excitation ideally suited to the 594 nm laser line. For stable signal generation in imaging and flow

cytometry, Alexa Fluor 594 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 594 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.

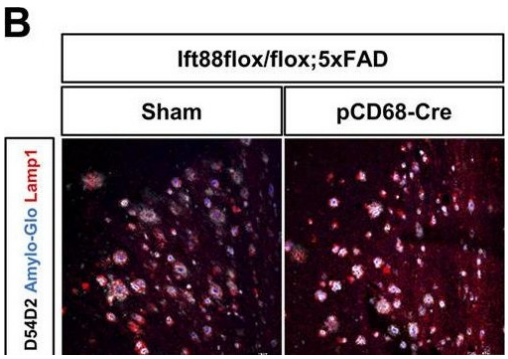
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594



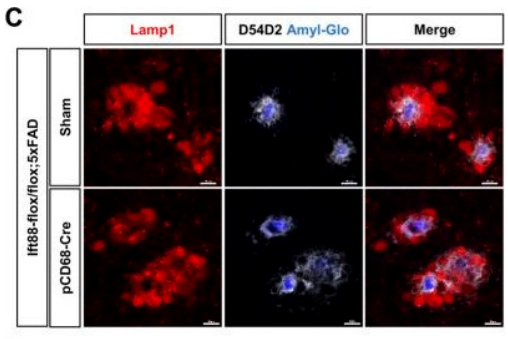
Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21209) in ICC /IF

Immunofluorescence analysis of Donkey anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 594 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 594 conjugate (Product # A-21209) 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: 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.



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21209) in ICC /IF

Increased A plaques and dystrophic neurites in the LS of Ift88-flox/flox; 5xFAD (10-month-old) 1 month after intracerebroventricular (ICV) administration of AAV-pCD68-Cre (A) A schematic drawing illustrates the stereotaxic injection of AAV-pCD68-Cre into the lateral ventricle of Ift88-flox/flox; 5xFAD mice and the examination of the septal region after 1 month. (B) Representative low magnification images of the LS for A plaques (D54D2, white), cored A plaques (Amylo-Glo, blue), and dystrophic neurites (Lamp1, red) in 5xFAD (sham) (10-month-old, n = 3, one male and two females) and Ift88-flox/flox; 5xFAD (10-month-old, n = 3, one male and two females), injected with AAV-pCD68-Cre. (C) Representative images of dystrophic neurites (Lamp1, red) adjacent to A plaques (D54D2, white). The cored A plaques were counter-stained using Amylo-Glo (blue). (D) Plots depict the number of A plaques marked with D54D2 and Amylo-Glo. The seeded Amylo-Glo-positive (50 µm) and growing cored plaques (200 µm) were measured. A plot depicts Lamp1-labeled dystrophic neurites as shown by the area ratio (Lamp1/D54D2) in each plaque in 5xFAD (sham) (10-month-old, n = 3, one male and two females) and Ift88-flox/flox; 5xFAD (10-month-old, n = 3, one male and two females), injected with AAV-pCD68-Cre. (E) Representative images of A plaque (A - Image collected and cropped by CiteAb under a CC-BY … Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/37942288), licensed under a CC BY license.



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735 References

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