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
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	-
Immunocytochemistry (ICC/IF)	1 µg/mL	-

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.

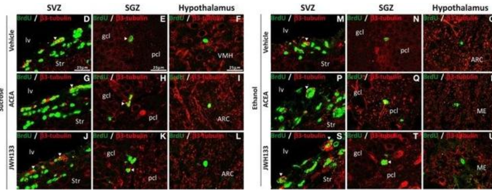
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over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

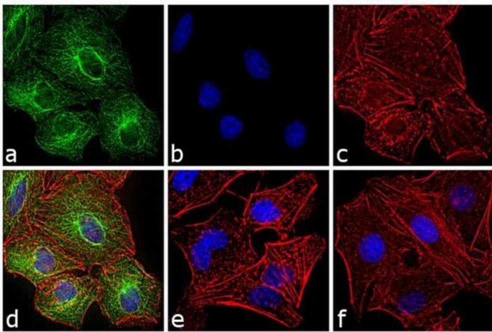
should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

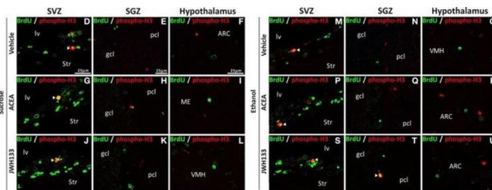
Product Images For Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21208) in IHC
Quantification of the proportion of cells labeled with BrdU that expressed 3-tubulin in the SVZ (A), SGZ (B), and hypothalamus (C) of vehicle, ACEA and JWH133-treated rats fed with sucrose and ethanol diets. Bars represent the percentage of labeled cells in each experimental group. (D–U) BrdU and 3-tubulin co-expression in the rat brain. High-resolution confocal laser scanning photomicrographs showing the labeling of BrdU (green) and 3-tubulin (red) in the SVZ, SGZ, and hypothalamus of vehicle, ACEA and JWH133-treated rats fed with sucrose (left panel) and ethanol (right panel) diets. The arrowheads indicate co-expression. Scale bars are included in the left-upper images. Image collected and cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fncel.2015.00379/abstract), licensed under a CC BY license.



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 IHC
Quantification of the proportion of cells labeled with BrdU that expressed phospho-H3 in the SVZ (A), SGZ (B), and hypothalamus (C) of vehicle, ACEA and JWH133-treated rats fed with sucrose and ethanol diets. Bars represent the percentage of labeled cells in each experimental group. (D–U) BrdU and phospho-H3 co-expression in the rat brain. High-resolution confocal laser scanning photomicrographs showing the labeling of BrdU (green) and phospho-H3 (red) in the SVZ, SGZ, and hypothalamus of vehicle, ACEA and JWH133-treated rats fed with sucrose (left panel) and ethanol (right panel) diets. The arrowheads indicate co-expression. Scale bars are included in the left-upper images. Image collected and cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fncel.2015.00379/abstract), licensed under a CC BY license.

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911 References

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Phasic stimulation in the nucleus accumbens enhances learning after traumatic brain injury. Cereb Cortex Commun (2022)

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