F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

Product Details	
Size	500 µg
Species Reactivity	Rabbit
Host/Isotype	Goat / 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_2534114

Applications	Tested Dilution	Publications
Immunocytochemistry (ICC/IF)	2 µg/mL	-

Product Specific Information

To minimize cross-reactivity, these goat anti-rabbit IgG (H+L) divalent F(ab')2 secondary antibodies have been affinity purified and cross-adsorbed against pooled human serum, mouse serum, mouse plasmacytoma/ hybridoma proteins, and purified human paraproteins. 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 are may be the presence of endogenous immunoglobulins.

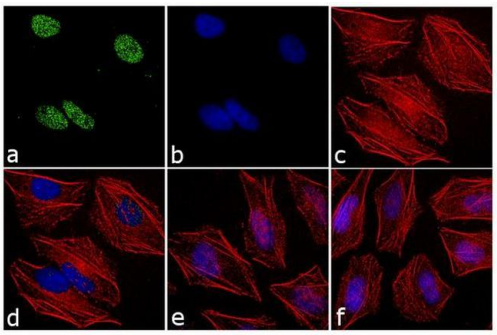
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over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

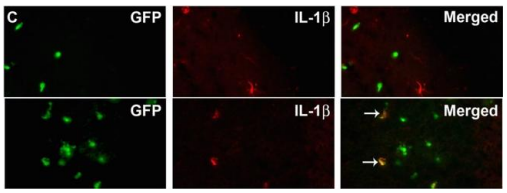
for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

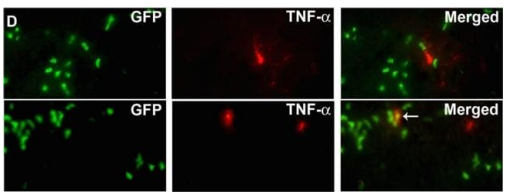
Product Images For F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11070) in ICC/IF
Immunofluorescence analysis of F (ab')2-Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488 (Product # A-11070) was performed using HeLa cells stained with PARP Rabbit Polyclonal Primary Antibody (Product # PA5-16452). 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 of rabbit primary antibody for 3 hours at room temperature. F (ab')2-Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488 (Product # A-11070) was used at a concentration of 2 µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of PARP in the nucleus (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.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11070) in IHC (PFA)
IL-1 and TNF- are expressed by macrophages and microglia. (A) Infiltrating GFP+ cells consist of Gr1+ granulocytes and Gr1- macrophages. (B) Double immunofluorescence for Gr1+ granulocytes and either IL-1 or TNF- showed no detectable co-expression 24 hours after pMCAO. Immunofluorescence detection of GFP and IL-1 (C) or TNF- (D) showed that these cytokines are expressed by resident GFP- microglia and infiltrating GFP+ macrophages (E) Immunofluorescence double staining confirmed the flow cytometry results by showing that IL-1 and TNF- are expressed by largely segregated subpopulations of cells. Very few IL-1+TNF-+ co-expressing cells were identified during microscopic analysis (E). Gr1+ granulocytes were visualized using Alexa Fluor® 594-conjugated goat anti-rat IgG, and IL-1+ and TNF-+ cells using both Alexa Fluor® 488-conjugated goat anti-rabbit and chicken anti-rabbit IgG. Scale bars: 50 m (A) 20 m (insert, A), 20 m (B-E). Image collected and cropped by CiteAb from the following publication (http://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-5-46), licensed under a CC BY license.



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641 References

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