Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 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_2535849

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
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)	4 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, these goat anti-rabbit IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. 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.

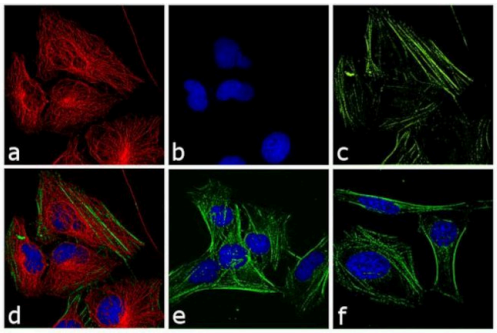
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow

cytometry, Alexa Fluor 555 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 555 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.

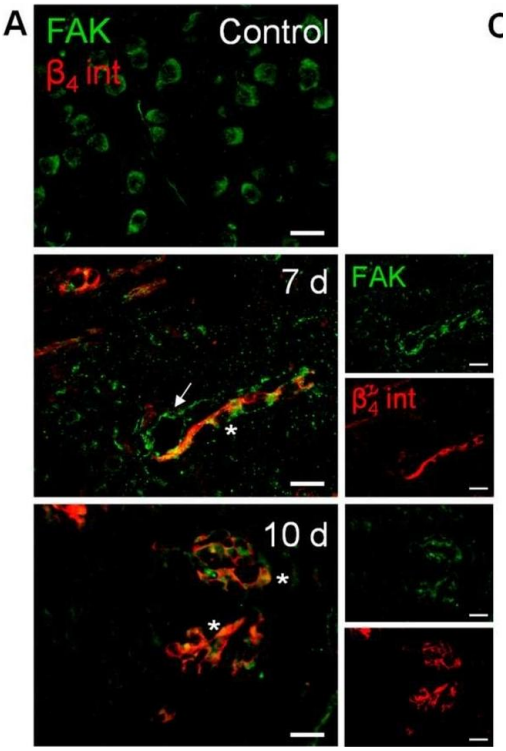
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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.

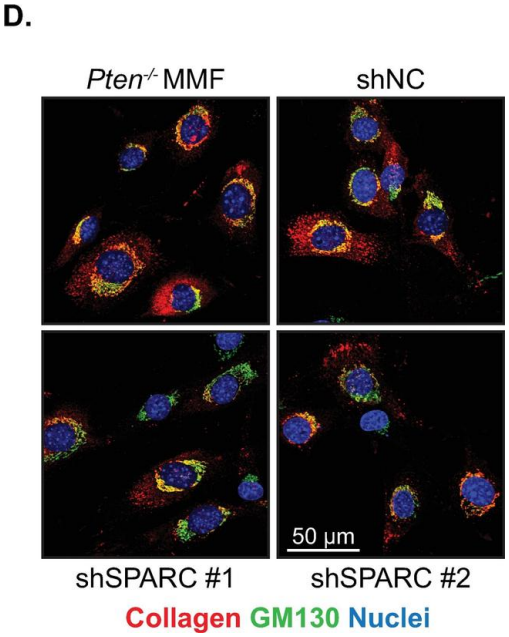
Product Images For Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21428) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555 was performed using HeLa cells stained with alpha Tubulin Rabbit Polyclonal Primary Antibody (Product # PA5-16891). 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 rabbit primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555 (Product # A-21428) 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.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21428) in ICC/IF
Adhesion and migration-associated signaling molecules are expressed in breast cancer (BC) brain metastases. The 4T1 cells or vehicle (controls) were inoculated in the carotid arteries of female Balb/c mice and hippocampal sections were analyzed after 5 h (h), 3, 7 and 10 days (d). (A) Double labelling with focal adhesion kinase (FAK, green) and 4 integrin (4 int, red) showed brain-resident FAK-positive cells, FAK-positive blood vessels feeding metastases (arrow) and co-labelling of FAK and 4 integrin in metastatic lesions (asterisks). Scale bar: 20 µm. (B) Quantification of co-labelled FAK and 4 integrin (FAK + 4 integrin)-positive cells per field revealed an increase from 7 to 10 d. (C) Ras-related C3 botulinum toxin substrate 1 (Rac1)-positive (yellow) BC cells were observed in lectin-positive (green) brain metastasis, which peaked at 7 d (magnified sections). Scale bar: 20 µm. (D) Semi-quantitative analysis revealed that Rac1 decreases from 7 to 10 d. (E) RhoA-positive (red) BC cells were observed in well-established metastasis, with malignant cells presenting nuclear translocation at 10 d (arrows). Nuclei (blue) were counterstained with Hoechst 33342. Scale bar: 20 µm. (F) Semi-quantitative analysis of RhoA immunoreactivity revealed a significant increase at 10 d. (G) Myosin light chain kinase (MLCK) (yellow) expression was observed in BC cells inside claudin-5-positive (r… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33671551), licensed under a CC BY license.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21428) in ICC/IF
SPARC knockdown does not affect collagen shuttling.(A) Western blot verifying SPARC knockdown in both the cell lysate and the culture medium, with GAPDH as a loading control. (B) Western blot for collagen I in the cell lysate and culture medium with SPARC knockdown. (C) Quantification of collagen expression in the culture medium with SPARC knockdown, normalized to Pten-/- MMF. n = 3+SD, with no significant difference observed between conditions. (D) Immunofluorescence images of MMF plated for 24 hours and cultured with 50 µg /mL ascorbic acid for 1 hour, then fixed and stained for collagen, GM130, and nuclei. (E) Quantification of collagen colocalization with GM130, determined by Manders' correlation coefficient and normalized to the Pten-/- parental condition. n = 3+SD. **p<0.01, ***p<0.001, ****p<0.0001 compared to the same cell line without ascorbic acid treatment. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33534863), licensed under a CC BY license.

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1796 References

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