Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 568
Excitation/Emission Max	579/603 nm
Immunogen	Gamma Immunoglobin
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2534017

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

Product Specific Information

These donkey anti-rabbit 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, rat, 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.

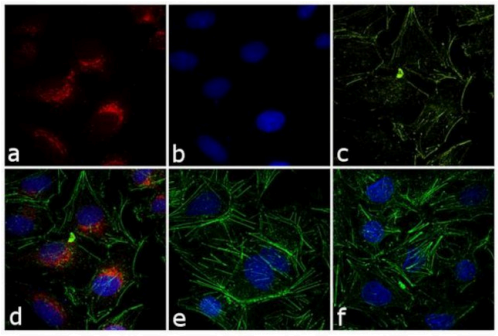
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright, orange/red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 dye is pH-insensitive 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 568 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.

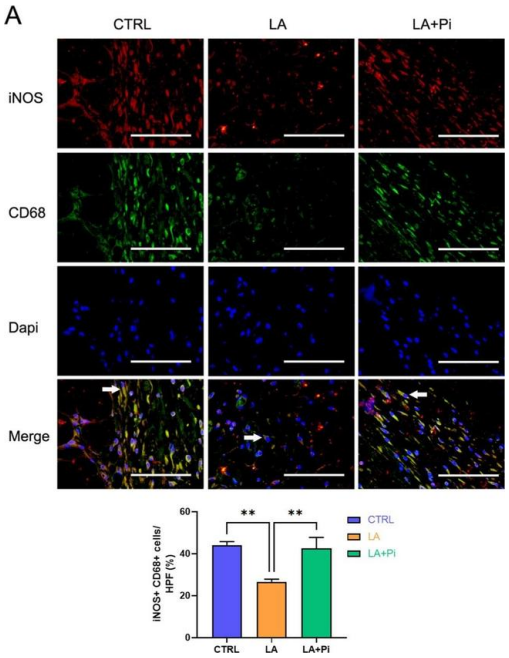
Product will be shipped at Room Temperature.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568



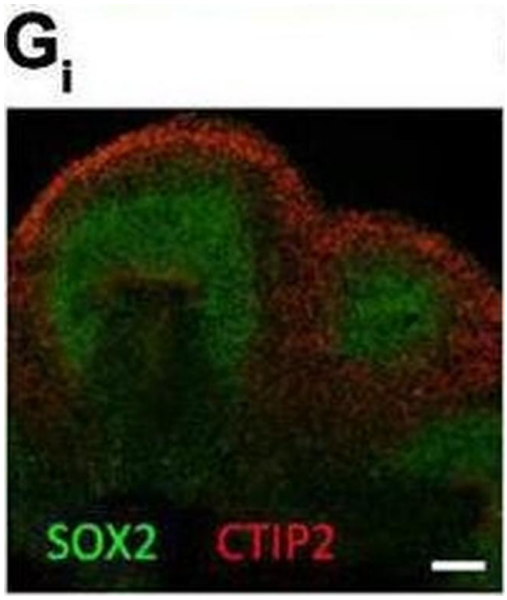
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10042) in ICC/IF

Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 568 (Product # A10042) was performed using HepG2 cells stained with alpha-1 antitrypsin Rabbit Polyclonal Primary Antibody (Product # PA5-16661). 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. Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 568 (Product # A10042) 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-1 antitrypsin 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) Highly Cross-Adsorbed Secondary Antibody (A10042) in ICC/IF

Lactate promotes the transition of wound macrophages to a reparative phenotype and accelerates wound healing in mice by activating PKM2. Eighteen mice were randomly divided into three intervention groups: the CTRL group (treated with vehicle, n = 6), the LA group (treated with 20 mM lactate, n = 6), and the LA+Pi group (treated with lactate + 1.2 µM PKM2 enzymatic inhibitor, n = 6). Immunofluorescence staining with iNOS + CD68 (A) or ARG1 + CD68 (B) was performed to analyze wound macrophages on Day 5 post-injury. Scale bar = 100 µm. n = 6. (C) The photographs of skin wounds and schematic diagram of comparison of wound area on Day 3 and Day 9. Scale bar = 5 mm. (D) Statistical results of relative wound area (fold of the wound on Day 3). # means the statistical difference between the LA and LA + Pi groups, * means the statistical difference between the LA and CTRL groups. (E) Relative wound areas on Day 9 (Fold of the wound on Day 3) were analyzed (n = 6). (F) Skin wound hematoxylin and eosin (HE) staining on Day 5. Scale bar = 400 µm. (G) Skin wound HE staining on Day 12. Data represents mean ± SEM, *p < 0.5, **p < 0.01, ##p < 0.01. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36439872), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10042) in ICC/IF

NCAM as a neuronal exosome marker in plasma. (A-C) Quantitative analysis of plasma EVs using flow cytometry. Dot plots of fluorescent intensity for plasma EVs stained with PE-CY7-labeled CD63 antibody. EVs were unstained (A) and stained with either isotype control (B) or CD63 antibody (C). (D) A18945 iPSC-derived neurons cultured for 6 weeks and immunostaining with mature neuronal markers (DAPI, MAP2, NeuN, and merged image). Images were taken using Zeiss LSM confocal microscope at ×63 magnification. Scale bar, 20 µm. (E) Representative images of cortical organoids at day 90 of differentiation. Scale bar, 1 mm. (F) The organoids express cortical layer marker BRN2 (also called POU3F2). The nuclei were stained with DAPI. Scale bar, 100 µm. (Gi) Markers for proliferating neural progenitors (SOX2) and cortical neuron marker CTIP2 (also known as BCL11B) with nuclei DAPI staining (Gii). (H) Western blot analysis of NCAM and CD63 from EVs released from iPSCs, iPSC-derived cortical neurons, and iPSC-derived brain organoids. EVs were isolated using SEC from the cell culture media of each sample, and equal EV particle numbers (6 × 108) were subject to immunoblotting with NCAM and CD63 antibodies. (I-L) Flow cytometry dot plots of fluorescent intensity for plasma EVs double-stained with DiI and NCAM antibody. EV samples were unstained (I), single stained with DiI (J), and double-stained with DiI and NCAM antibody… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35655952), licensed under a CC BY license.

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1574 References

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