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
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
Immunohistochemistry (IHC)	1-10 µg/mL	-
Immunocytochemistry (ICC/IF)	4 µg/mL	-

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.

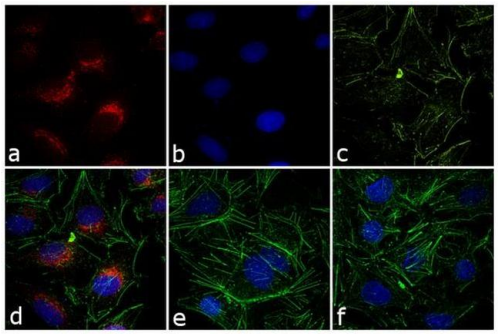
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright, orange /red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 dye is pH-insensitive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

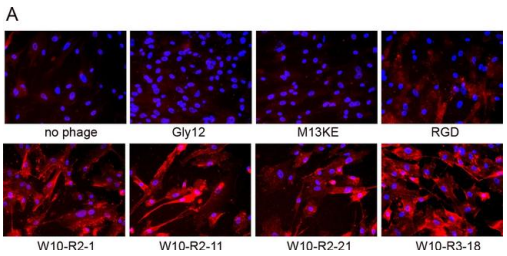
Product will be shipped at Room Temperature.

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

Binding of peptide display phages to W10 embryonic progenitor cell line.(A) Immunofluorescent detection of bound phages. Cells were incubated with 2×1010 phage particles for 2 h at 37°C; unbound phages were removed by washing and cells were fixed and permeabilized. Bound phages were detected by immunocytochemistry using rabbit anti-phage antibody and Alexa568-conjugated goat anti-rabbit antibody. Cell nuclei were stained using DAPI. (B) Quantitation of peptide phage cell binding. 2×1010 pfu of each candidate or controls (RGD, Gly12 and empty phage M13KE) phages were assessed for binding on 1×105 W10 progenitor cells for 2 h at 37°C. Cell associated phages were recovered from cell lysates and quantified by titration. Protein in cell lysates was measured by microBCA assay. The relative binding factor (BF) is calculated as peptide phage recovery (percentage of input) relative to M13KE control phage recovery (percentage of input). Values are from triplicate experiments and shown as mean ± standard deviation. BFs for the 4 W10 peptide phage were statistical significant from the control M13KE phage (ANOVA with Dunnett's multiple comparison tests; p values: *: <0.05 and **: <0.01). BFs for RGD and Gly12 were not statistically significant. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0058200), licensed under a CC BY license.

987 References

Engineered neuronal microtissue provides exogenous axons for delayed nerve fusion and rapid neuromuscular recovery in rats. Bioact Mater (2022)

Crosstalk between cancer and different cancer stroma subtypes promotes the infiltration of tumorassociated macrophages into the tumor microenvironment of oral squamous cell carcinoma. Int J Oncol (2022)

Partial and complete loss of myosin binding protein H-like cause cardiac conduction defects. J Mol Cell Cardiol (2022)

Glutamate transporters EAAT2 and EAAT5 differentially shape synaptic transmission from rod bipolar cell terminals. Eneuro (2022)

sEVsRVG selectively delivers antiviral siRNA to fetus brain, inhibits ZIKV infection and mitigates ZIKV-induced microcephaly in mouse model. Mol Ther (2022)