Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotope	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_141607

Applications	Tested	Dilution	Published
Immunohistochemistry (IHC)	✓	1-10 µg/mL	6 Publications
Immunocytochemistry (ICC)	✓	0.2 µg/mL	17 Publications
Immunohistochemistry (Paraffin) (IHC (P))	-	1:1000	3 Publications
Immunohistochemistry (Frozen) (IHC (F))	-	1:500	5 Publications
Flow Cytometry (Flow)	-		1 Publication
Miscellaneous PubMed (Misc)	-		36 Publications
Immunofluorescence (IF)	✓	1:2000	

Product Specific Information

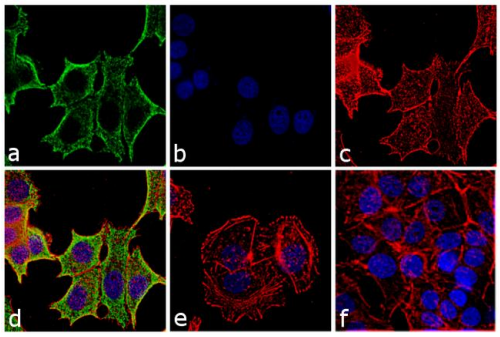
To minimize cross-reactivity, these donkey anti-mouse IgG whole antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rat, and sheep serum proteins. Cross-adsorption or pre-adsorption is a purification step to increase 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 488 dye is a bright, green-

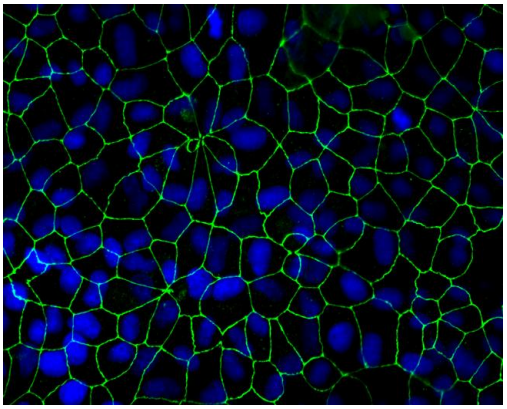
fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological 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 Images For Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21202) in IF
Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate (Product # A-21202) was performed using MCF-7 cells stained with Cytokeratin 19 Mouse Monoclonal Antibody (Product # MA5-12613). 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 mouse primary antibody (1:250 dilution) for 3 hours at room temperature. Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate was used at concentration of 0.2 µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of cytokeratin 19 in the membrane (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.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21202) in IF
Immunofluorescent detection of Zo-1 in MDCK cells. Confluent monolayers were fixed in 50%methanol/50%Acetone, blocked for at least 30 minutes in 1% BSA then incubated 2 hours with a Zo-1 monoclonal antibody (Product # 33-9100) at 5 µg/mL, washed, then incubated 1 hour with Alexa Fluor 488 conjugated Donkey anti-Mouse secondary antibody (Product # A-21202) at a dilution of 1:2000. Cells were counterstained with DAPI (blue). Coverslips were mounted with Prolong Gold Antifade reagent (Product # P36930) and imaged at 40X. Images generated by Joell Solan in Paul Lampe Lab at the Fred Hutchinson cancer Research Center.

68 References

Immunohistochemistry (6)

Frontiers in surgery

The Identification of Three Cancer Stem Cell Subpopulations within Moderately Differentiated Lip Squamous Cell Carcinoma.

"A21202 was used in immunohistochemistry to identify and characterize cancer stem cells in moderately differentiated lip squamous cell carcinoma"

Authors: Ram R,Brasch HD,Dunne JC,Davis PF,Tan ST,Itinteang T

Species
Not Applicable

Dilution
1:500

Year
2019

Journal of neuroinflammation

Microglia-derived IL-1 contributes to axon development disorders and synaptic deficit through p38-MAPK signal pathway in septic neonatal rats.

"A21202 was used in immunohistochemistry to test if interleukin-1b derived from microglia affects myelination and axon development in lipopolysaccharide-treated cells"

Authors: Han Q,Lin Q,Huang P,Chen M,Hu X,Fu H,He S,Shen F,Zeng H,Deng Y

Species
Not Applicable

Dilution
1:200

Year
2017

View more IHC references on thermofisher.com

Immunocytochemistry (17)

Spermatogenesis

The nuclear form of glutathione peroxidase 4 colocalizes and directly interacts with protamines in the nuclear matrix during mouse sperm chromatin assembly.

"A-21202 was used in immunocytochemistry to test if nGPx4 directly interacts with protamines by transiently sharing a nuclear matrix localization."

Authors: Puglisi R,Maccari I,Pipolo S,Mangia F,Boitani C

Species
Not Applicable

Dilution
1:500

Year
2019

Nature

Cell diversity and network dynamics in photosensitive human brain organoids.

"A21202 was used in immunocytochemistry to compare the gene expression in individual cells isolated from human brain organoids"

Authors: Quadrato G,Nguyen T,Macosko EZ,Sherwood JL,Min Yang S,Berger DR,Maria N,Scholvin J,Goldman M,Kinney JP,Boyden ES,Lichtman JW,Williams ZM,McCarroll SA,Arlotta P

Species
Not Applicable

Dilution
1:1200

Year
2017

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IHC (P) (3) **IHC (F) (5)** **Flow (1)** **Misc (36)**