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

Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
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_2535853

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	1-10 µg/mL	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-

Product Specific Information

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

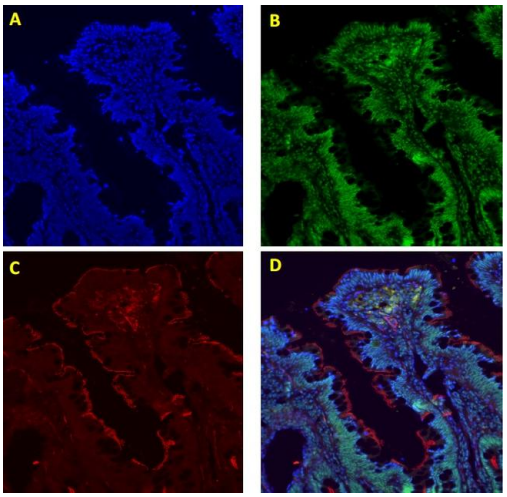
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive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

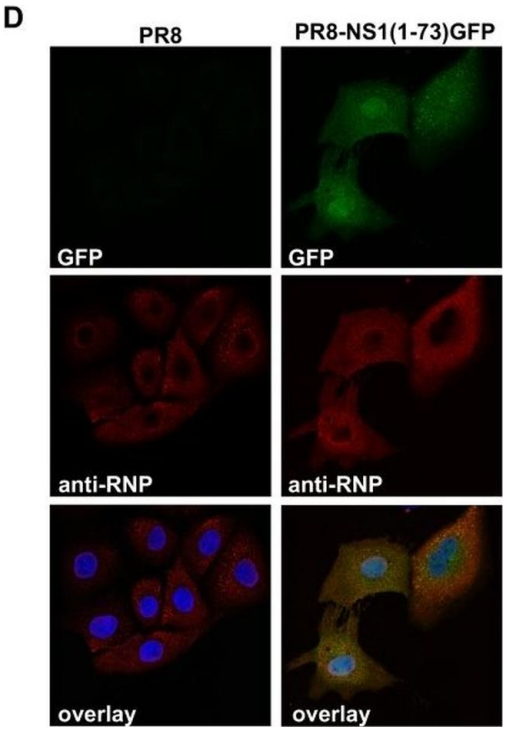
should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

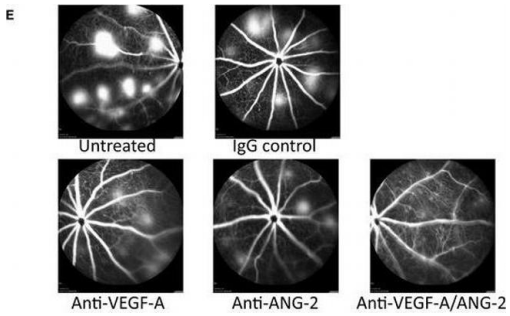
Product Images For Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21432) in ICC/IF
Immunofluorescence analysis of H3K4me3 (green) and ZO-1 (red) in Rhesus Macaques. Cells were stained with a recombinant monoclonal H3K4me3 antibody (Product # 703849) and monoclonal ZO-1 antibody (Product # 33-9100) followed by incubation with secondary Alexa Fluor &reg 488 conjugate antibody (Product # A11034) (panel b: green) and secondary secondary Alexa Fluor &reg 555 conjugate antibody (Product # A21432) (panel c: red) . Nuclei (panel a: blue) were stained with DAPI. Panel d is a merged image of panels a, b and c. Images were taken on EVOS2 at 20X magnification. Data courtesy of J. Arredondo and S. Dandekar at UC Davis.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21432) in ICC/IF
In vitro characterization of the PR8-NS1(1–73)GFP virus.(A) Schematic representation of the promoters and coding sequences of the pHW-NS1(1–73)Dmd-GFP-NEP plasmid used to generate the reporter GFP influenza virus. (B) Multi-cycle growth kinetics. MDCK cells were infected in duplicate with a MOI of 0.001 of wild type PR8 virus or PR8-NS1(1–73)GFP virus in the presence of TPCK-trypsin. At the indicated time points after infection, the viral titer in the supernatant (50 l sample) was determined by TCID50 analysis. The graph shows the mean with the standard error of each data point. For the wild type PR8 virus, one of the duplicate samples of the 48 h time point was excluded due to technical failure. (C) Plaques of PR8 and PR8-NS1(1–73)GFP virus were visualized on day three after infection of MDCK cells by immunostaining with an M2e-specific monoclonal antibody. (D) Confocal microscopy analysis of MDCK cells infected with wild type PR8 or PR8-NS1(1–73) GFP virus (MOI 1). Twenty four hours after infection the cells were fixed and stained with anti-RNP (red; middle panel) and Hoechst (blue). The GFP signal is shown in green (top panel). An overlay of the three colors is shown in the bottom panel. (E) MDCK cells were infected with a MOI of 1 of PR8-NS1(1–73)GFP virus or wild type PR8 virus, or were not infected (NI). After 24 h, lysates were prepared and the proteins were visualized b… Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0121491), licensed under a CC BY license.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21432) in IHC (F)
Reduction of vessel leakiness and lesion number by combined neutralization of VEGF-A and ANG-2 in the JR5558 mice using FFA(A) Study schematic. Baseline FFA was carried out at P44-45. Antibody injections were given IP on P46 and P53. Post-treatment FFA was at P60 and tissue harvest at P61. (B) Bar/scatter graph of baseline lesion numbers. Lesions were counted in all mice prior to study start. Combined total lesions from left and right eyes were calculated, and then, animals were assigned to treatment groups ensuring no statistically significant differences between groups (P > 0.05). (C, D) Bar/scatter graphs showing numbers of spontaneously occurring lesions (C) and area by fluorescence angiography (D) after two weekly doses of antibody (IgG, anti-VEGF-A, anti-ANG-2 at 5 mg/kg IP, and anti-VEGF-A/ANG-2 at 10 mg/kg IP) followed by analysis a week after the last treatment. (E) Representative examples of fluorescence fundus angiograms from the untreated (top left), IgG control (top middle; 10 mg/kg), anti-VEGF-A (bottom left; 5 mg/kg), anti-ANG-2 (bottom middle; 5 mg/kg), and anti-VEGF-A/ANG-2 (bottom right; 10 mg/kg) groups. Data information: SEM is shown as error bars with n = 9–10 animals (B) or n = 19–20 (C, D) eyes per group and significance indicated by asterisks using ANOVA (B: P > 0.05; C: P < 0.0001; D: P&nbs… Image collected and cropped by CiteAb from the following publication (https://onlinelibrary.wiley.com/doi/10.15252/emmm.201810204), licensed under a CC BY license.

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495 References

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Neuronal SH2B1 attenuates apoptosis in an MPTP mouse model of Parkinson's disease via promoting PLIN4 degradation. Redox Biol (2022)

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