Goat anti-Rabbit IgG (H+L) Highly 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_2535850

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	-	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)	1-10 µg/mL	-
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 bovine IgG, goat IgG, mouse IgG, rat IgG, 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.

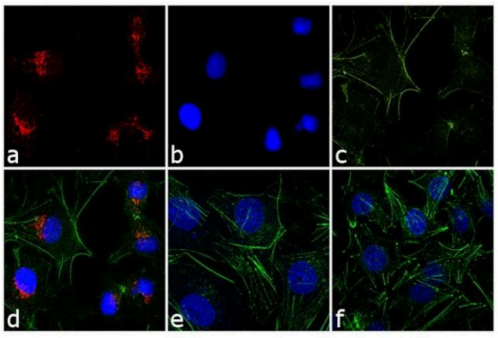
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive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

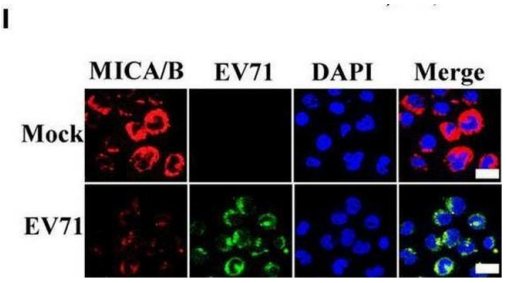
Product will be shipped at Room Temperature.

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



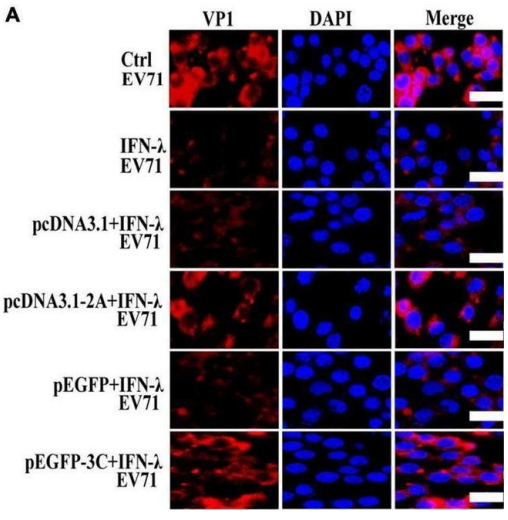
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21429) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555 (Product # A-21429) 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. Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555 (Product # A-21429) 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 (A-21429) in ICC/IF

EV71+IECs express lower levels of MICA/B and higher levels of PD-L1 and are less susceptible to iNK and CD3+iIELs lysis. (A) The intestinal intraepithelial lymphocytes from the C57BL/6 mouse were separated and extracted, and the ratio of CD3+iIELs and NK1.1+iNK cells was analyzed by flow cytometry. (B) HT29 cells were infected with EV71 at an MOI of 1 for 48 h. LDH was applied to analyze the susceptibility of EV71-HT29 and EV71+HT29 to NK-92 cell lysis at 6 h at the indicated E:T ratios. (C,E) The intestinal intraepithelial lymphocytes were prepared to enrich iNK or CD3+iIELs using the MojoSort™ Mouse NK Cell Isolation Kit or the MojoSort™ Mouse CD3 T Cell Isolation Kit by MACS. Cells were stained with anti-NK1.1 antibody or anti-CD3 antibody. (D,F) The h-SCARB2-MC38 cells were infected with EV71 at an MOI of 1 for 48 h. LDH was applied to analyze the susceptibility of EV71-h-SCARB2-MC38 and EV71+h-SCARB2-MC38 to iNK or CD3+iIELs cell lysis on 10 h at the indicated E:T ratios. (G,H) HT29 cells were infected with EV71 at an MOI of 1. The membrane expression levels of MICA/B or PD-L1 were detected at 48 hpi by FACS. (I,J) At 48 hpi, cells were fixed and stained with anti-EV71 (green) antibodies, anti-MICA /B or PD-L1 (red) antibodies, and DAPI (blue) and examined by a confocal microscope. Mock was cells without infection. Merged images of the different channels were shown. (K,L) The expression levels of NKG2D or PD… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35185830), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21429) in ICC/IF

The 2Apro and 3Cpro of EV71 antagonize the antiviral function of IFN- and inhibit the expression level of IFN- induced by poly(I:C). HT29 cells were transfected with pcDNA3.1-2A (control plasmid: pcDNA3.1) or p-EGFP-3C (control plasmid: p-EGFP). After transfection 24 h, cells were treated with IFN- (10 ng/ml) 6 h before infection. Then cells were infected with EV71 at an MOI of 1. Cells and culture mediums were collected at 24 hpi. (A) The protein expression level of VP1 was determined by incubating with anti-EV71 VP1 antibody, followed by staining with Alexa Fluor 555-conjugated secondary antibody. (B) The mRNA expression levels of VP1 were determined by qRT-PCR. (C) Cell culture medium was prepared for virus titer analysis. Control was cells with infected only. IFN- group was cells infected and using IFN- pretreatment. (D,E) HT29 cells were transfected with pcDNA3.1-2A (pcDNA3.1) or p-EGFP-3C (p-EGFP), and after transfection 24 h, cells were treated with poly(I:C) (4 µg/ml). Cells and culture mediums were collected 24 h after stimulation. The protein expression levels of IFN-1 and IFN-2 were determined by ELISA, and the mRNA expression levels were determined by qRT-PCR. Control was cells without any treatment. PIC group was cells stimulated only by poly(I:C). Dates were presented as mean ± SD (n = 3 independent experiments, *P < 0.05, **P < 0.01 and ***P < 0.001). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35185830), licensed under a CC BY license.

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848 References

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