

Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633

| Product Details | |
|-------------------------|--|
| Size | 1 mg |
| Species Reactivity | Rabbit |
| Host/Isotype | Goat / IgG |
| Class | Polyclonal |
| Type | Secondary Antibody |
| Conjugate | Alexa Fluor™ 633 |
| Excitation/Emission Max | 631/650 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_2535732 |

| Applications | Tested Dilution | Publications |
|--|------------------|---------------|
| Western Blot (WB) | 1:5,000-1:10,000 | - |
| Immunohistochemistry (IHC) | - | 0 Publication |
| Immunohistochemistry (Paraffin) (IHC (P)) | 1:2,000 | 0 Publication |
| Immunohistochemistry (PFA fixed) (IHC (PFA)) | - | 0 Publication |
| Immunohistochemistry (Frozen) (IHC (F)) | - | 0 Publication |
| Immunocytochemistry (ICC/IF) | 1:500-1:2,000 | 0 Publication |
| Flow Cytometry (Flow) | 1-10 µg/mL | - |
| Miscellaneous PubMed (Misc) | - | 0 Publication |

Product Specific Information

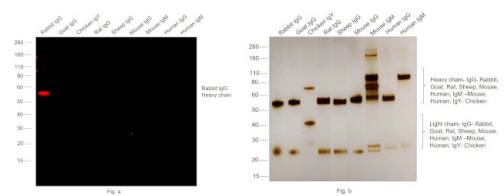
To minimize cross-reactivity, these goat anti-rabbit IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting 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 may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 633 dye is a bright, far-red-fluorescent dye with excitation ideally suited to the 633 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 633 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 633 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.

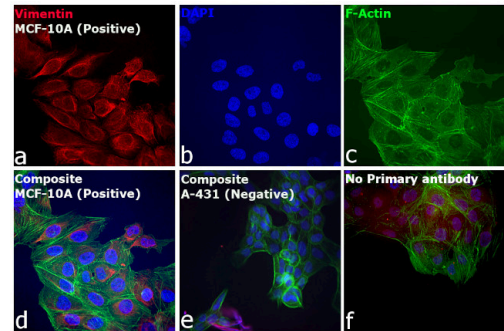


Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21071)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG. A band at ~55 kDa corresponding to Rabbit IgG Heavy Chain was observed in Rabbit IgG but not in other species using Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633 (Product # A-21071) in Western Blot. {RE}

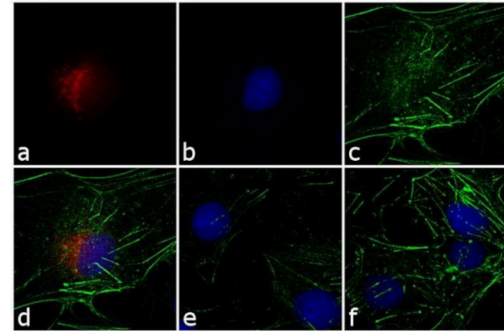
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21071) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 633 (Product # A-21071) was performed using MCF 10A (positive model) and A-431 (negative model) in cells stained with Vimentin Polyclonal antibody (Product # PA5-27231). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 2% BSA for 1 hour and labeled with 1:500 of primary antibody overnight at 4C. Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 633 (Product # A-21071, 1: 2000) in 0.1% BSA in PBS for 1 hour at room temperature, was used for detection of Vimentin in the cytoskeleton (Panel a: Red). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:500) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in A-431 (negative model for Vimentin) due to no primary antibody binding (Panel e). Non-specific staining was not observed with secondary antibody alone (panel f). The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR) and externally deconvoluted (D.Sage et al./Methods 115 (2017) 28–41).



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-21071) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 633 (Product # A-21071) 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 633 (Product # A-21071) 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.



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