Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 647
Excitation/Emission Max	650/671 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_2536183

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

Product Specific Information

To minimize cross-reactivity, these donkey anti-rabbit IgG whole antibodies have been affinity-purified and show a published cross-reactivity to rat 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.

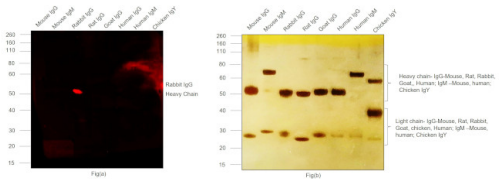
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 647 dye is a near-infrared-fluorescent dye with excitation ideally suited to the 647 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 647 dye is pH-insensitive over 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 647 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.

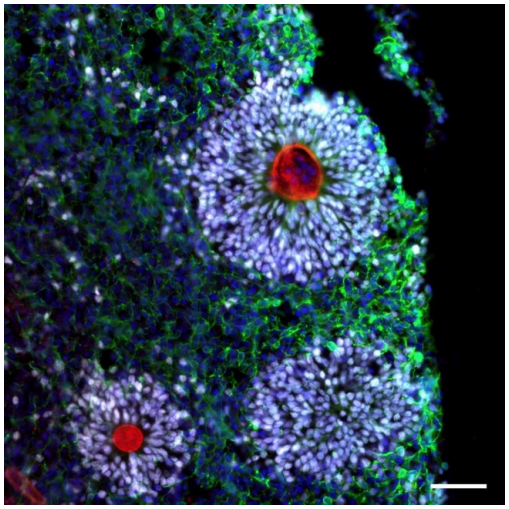
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because 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.

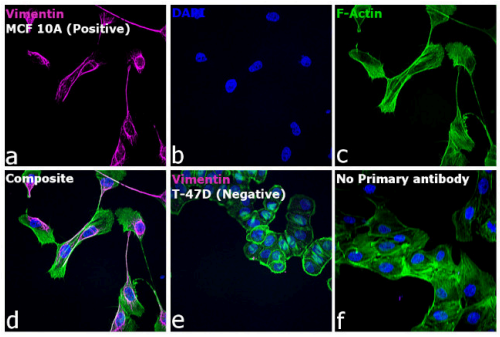
Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573)
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 ~50 kDa corresponding to Rabbit IgG Heavy Chain was observed in Rabbit IgG but not in other species using Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Product # A-31573) in Western Blot.Relative expression. {RE}



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573) in IHC (F)
Immunofluorescent analysis of ZO-1 (red) and SOX2 (grey) in human iPSC-derived forebrain organoids derived at Day 40. The organoids were fixed with 4% PFA for 1 hour at room temperature, followed by incubation with 30% sucrose solution overnight at 4°C. The organoids were then embedded in OCT and cryosectioned at 5 µm, permeabilized with 0.2% Triton X-100 for 20 min, and blocked with 10% donkey serum in PBS for 30 min at room temperature. Organoid slices were stained with a Mouse ZO-1 monoclonal antibody (red; Product # 33-9100) at a dilution of 1:500, a Rabbit SOX2 polyclonal antibody (grey; Product # PA1-094X) at a dilution of 1:500, and a Chicken MAP2 polyclonal antibody (green) at a dilution of 1:1000 in blocking buffer overnight at 4°C, and then incubated with Donkey anti-Mouse Alexa Fluor 568 (Product # A10037), Donkey anti-Rabbit Alexa Fluor 647 (Product # A31573), and Donkey Anti-Chicken Alexa Fluor 488 at a dilution of 1:1000 as well as DAPI (blue; 1: 25000) in blocking solution at room temperature for 1 hour. Images were taken at 20X magnification. Scale bar: 50 µm. Data courtesy of Dr. Zhexing Wen at Emory University.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A-31573) in ICC/IF
Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Product # A-31573) was performed using MCF 10A (positive model) and T-47D (negative model) 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 1% BSA for 1 hour and labeled with 2 µg/mL primary antibody for 3 hours at room temperature. Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Product # A-31573, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Vimentin in the cytoplasm (Panel a: pink). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:300) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in T-47D (negative model for vimentin) due to no primary antibody binding (Panel e). Nonspecific 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).

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2823 References

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