

Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 680
Excitation/Emission Max	681/704 nm
Immunogen	Gamma Immunoglobulin
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2534018

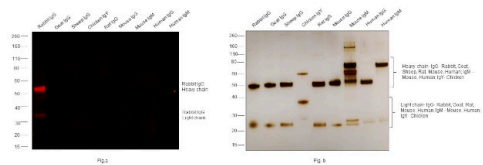
Applications	Tested Dilution	Publications
Western Blot (WB)	1:5,000-1:50,000	0 Publication
Immunocytochemistry (ICC/IF)	1:500-1:2,000	-
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

This secondary antibody is designed for fluorescent Western blot detection on various near-infrared fluorescence instruments. This antibody can be used for multi-color and multiplexing detection when using other antibodies conjugated to compatible Alexa Fluor™ dyes and wavelengths. Other applications of this antibody include immunofluorescent and fluorescent imaging applications when using instrumentation with appropriate excitation and detection capabilities. This antibody shows minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep serum proteins.

Product will be shipped at Room Temperature.

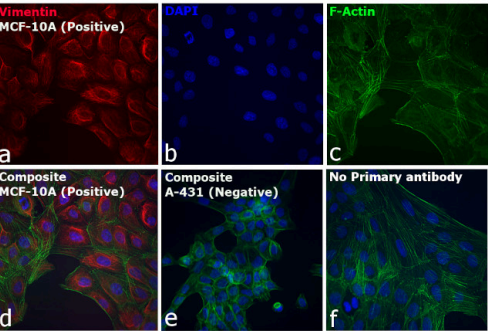
Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10043)
Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG. Bands at ~55 and 25 kDa corresponding to Rabbit IgG Heavy and Light Chain were observed in Rabbit IgG but not in other species using Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A10043) in Western Blot. {RE}

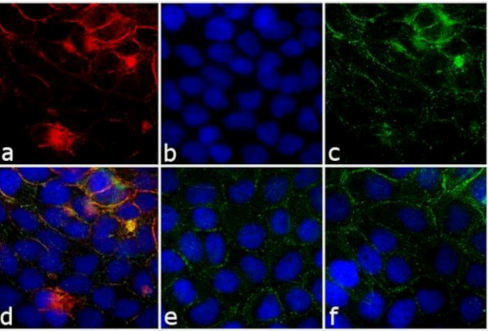
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A10043) in ICC/IF

Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A10043) 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 dilution of primary antibody overnight at 4C. Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 680 (Product # A10043, 1:2000 dilution) 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 (A10043) in ICC/IF

Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 680 was performed using A-431 cells stained with EGFR (EP38Y) Rabbit Monoclonal Primary Antibody (Product # MA5-14485). 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 Rabbit primary antibody (1:250 dilution) for 3 hours at room temperature. Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 680 (Product # A10043) 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 EGFR in the membrane (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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