Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488

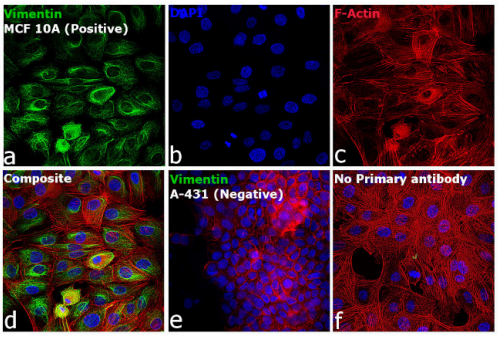
Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 488
Excitation/Emission Max	493/518 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	proprietary buffer, pH 6.5
Contains	0.016% Bromonitrodioxane, 0.016% Methylisothiazolone
Storage conditions	4° C, store in dark
RRID	AB_2762833

Applications	Tested Dilution	Publications
Western Blot (WB)	0.1-0.4 µg/mL	0 Publication
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	1:2,000	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

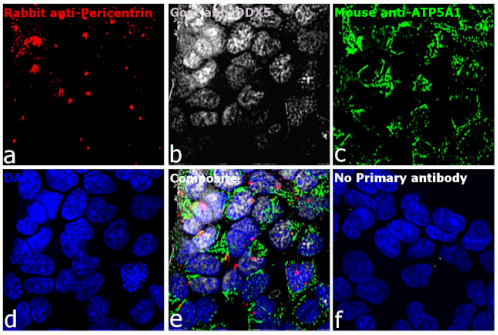
To minimize cross-reactivity, the donkey anti-Rabbit IgG whole antibodies have been cross-adsorbed against non-immunoglobulin rabbit serum proteins, IgG from bovine, goat, chicken, guinea pig, hamster, horse, sheep, mouse, rat, and human. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in less background staining and cross-reactivity. 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. Further passages through additional columns result in highly cross-adsorbed preparations of secondary antibody. The benefits of these extra steps are apparent in multiplexing /multicolor-staining experiments 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. 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. Specificity: This antibody binds to heavy chains on rabbit IgG and light chains on all rabbit immunoglobulins. This antibody does not bind non-immunoglobulin rabbit serum proteins or IgG from bovine, goat, chicken, guinea pig, hamster, horse, sheep, mouse, rat, or human.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32790) in ICC/IF

Immunofluorescence analysis of Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32790) was performed using MCF 10A (positive model) and A-431 (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™ Plus 488 (Product # A32790, 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: green). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor™ 647 Phalloidin (Product # A22287, 1:4000) (Panel c: red). 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). 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).

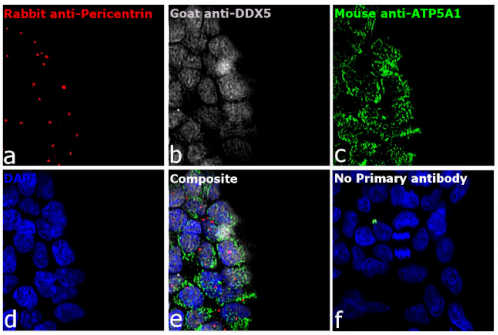


Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32790) in ICC/IF

Immunofluorescence analysis of A32790, A32816 and A32787 was performed using primary antibodies against Pericentrin (Product # PA5-53498), DDX5 (Product # PA1-31019) and ATP5A1 (Product # 43-9800) in 70% confluent log phase HEK 293 cells. The cells were fixed with 4% Paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 2% BSA, then incubated with the primary antibodies at 1:100 dilution each at 4 degree celsius overnight. The cells were then incubated with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32790), Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 555 (Product # A32816) and Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647 (Product # A32787) at 1:2000 dilution each in 0.1% BSA at room temperature for 45 minutes. 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). The specific centrosomal, nuclear and mitochondrial localization of Pericentrin (Panel a), DDX5 (Panel b) and ATP5A1 (Panel c) in the respective channels alone shows the specificity of all the 3 secondary antibodies used. Nuclei (Panel d) were stained with Hoechst33342 (Product # H1399). Panel e is the composite of Panels a-d, showing co-localisation. Panel f is control cells with

Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32790) in ICC/IF

Immunofluorescence analysis of A32790, A32860 and A32744 was performed using primary antibodies against Pericentrin (Product # PA5-53498), DDX5 (Product # PA1-31019) and ATP5A1 (Product # 43-9800) in 70% confluent log phase HEK 293 cells. The cells were fixed with 4% Paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 2% BSA, then incubated with the primary antibodies at 1:100 dilution each at 4 degree celsius overnight. The cells were then incubated with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32790), Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A32860) and Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594 (Product # A32744) at 1:2000 dilution each in 0.1% BSA at room temperature for 45 minutes. 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). The specific centrosomal, nuclear and mitochondrial localization of Pericentrin (Panel a), DDX5 (Panel b) and ATP5A1 (Panel c) in the respective channels alone shows the specificity of all the 3 secondary antibodies used. Nuclei (Panel d) were stained with Hoechst33342 (Product # H1399). Panel e is the composite of Panels a-d, showing co-localisation. Panel f is control cells with

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391 References

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