

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

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / 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% Methylisothiazolone, 0.016% Bromonitrodioxane
Storage conditions	4°C, store in dark
RRID	AB_2633275

Applications	Tested Dilution	Publications
Western Blot (WB)	0.1-0.4 µg/mL	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)	1-10 µg/mL	0 Publication
Flow Cytometry (Flow)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, the goat anti-mouse IgG whole antibodies have been pre cross-adsorbed against bovine IgG, goat IgG, rabbit IgG, rat IgG, human IgG, and human serum. 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.

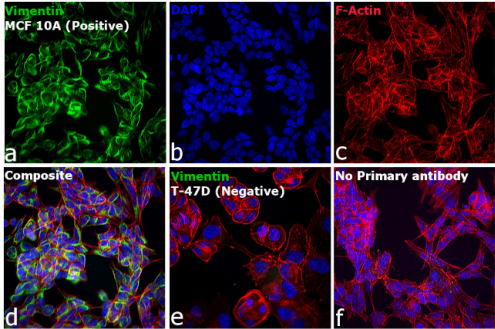
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage,

thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically.

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

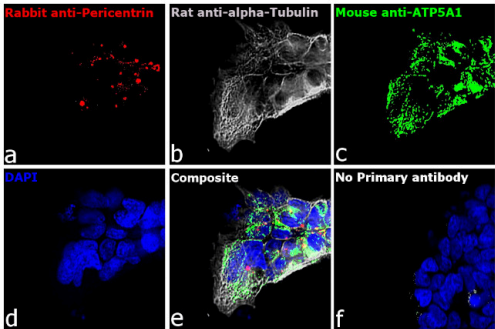
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32723) in ICC/IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32723) was performed using MCF 10A (positive model) and T-47D (negative model) cells stained with Vimentin Monoclonal Antibody (V9), eBioscience™ (Product # 14-9897-82). 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. Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32723, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Vimentin in the cytoskeleton (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 T-47D (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).



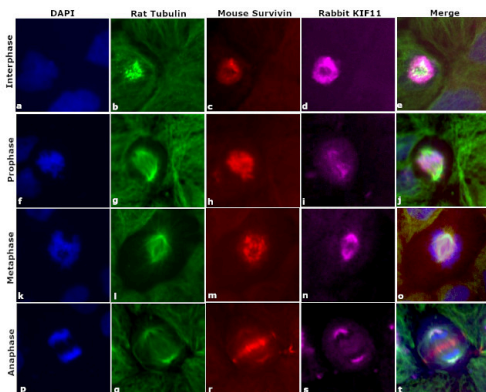
Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32723) in ICC/IF

Immunofluorescence analysis of A32734, A-11081 and A32723 was performed using primary antibodies against Pericentrin (Product # PA5-53498), alpha Tubulin (Product # MA1-80017) 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 Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 680 (Product # A32734), Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Product # A-11081) and Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32723) 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, cytoskeletal and mitochondrial localization of Pericentrin (Panel a), Tubulin (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 no primary antibody.



Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32723) in ICC/IF

Immunofluorescence analysis of A-32723, A-11081 and A-32734 was performed using primary antibodies against Survivin (Product # 14-9176-82), alpha Tubulin (Product # MA1-80017) and KIF11 (Product # MA5-32792) in 70% confluent log phase Caco-2 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 Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32723), Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546 (Product # A-11081) and Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 680 (Product # A-32734) 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 translocation of each protein during each stage of mitosis is visible in the respective channel alone (Tubulin-green), (KIF11-Magenta) and Survivin-Red). This shows the specificity of all the 3 secondary antibodies used. Nuclei (Panel d) were stained with Hoeschst (Product # P36962). Panels e,j,o and t are merged images showing co-localisation.



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1475 References

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NEXMIF overexpression is associated with autism-like behaviors and alterations in dendritic arborization and spine formation in mice. *Front Neurosci* (2025)

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