Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594

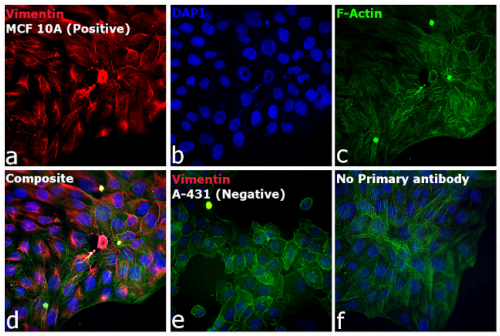
Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 594
Excitation/Emission Max	590/618 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	proprietary buffer, pH 6.5
Contains	0.016% Methylisothiazolone, 0.016% Bromonitrodioxane
Storage conditions	4° C, store in dark
RRID	AB_2762824

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

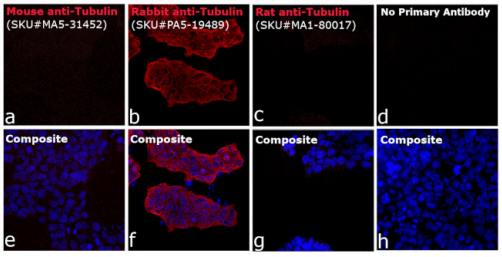
To minimize cross-reactivity, the goat anti-rabbit IgG whole antibodies have been cross-adsorbed against IgG from human, mouse and rat. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in less background staining and cross-reactivity. The secondary 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.^M
^M
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^M
^M
Specificity: This antibody binds to whole molecule rabbit IgG and light chains on other rabbit immunoglobulins. This antibody does not bind non-immunoglobulin serum proteins from human, mouse and rat. It has been pre-adsorbed for minimal cross-reactivity with IgG from human, mouse and rat sources.

Product Images For Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594



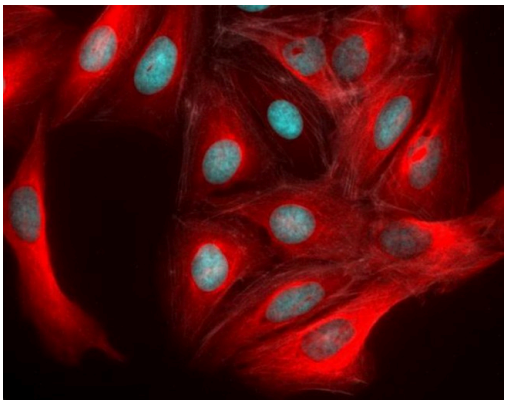
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32740) in ICC/IF

Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 (Product # A32740) 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. Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 (Product # A32740, 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: Red). 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 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 (A32740) in ICC/IF

Immunofluorescence analysis of A32740 was performed using anti-alpha tubulin antibodies 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 primary antibodies at 1:100 dilution 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 594 (Product # A32740) at 1:2000 dilution 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). Cytoskeletal localization of alpha-tubulin was observed only in cells stained with Rabbit alpha-Tubulin antibody (Product # PA5-19489) (Panels b and d), and not in the cells stained with Mouse alpha-Tubulin antibody (Product # MA5-31452) (Panels a and e) or Rat alpha-Tubulin antibody (Product # MA1-80017) (Panels c and g), demonstrating the host specific reactivity of A32740. Nuclei (blue) were stained with Hoechst33342 (Product # H1399). Panels d and h represent control cells with no primary antibody.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32740) in ICC/IF

Immunofluorescent analysis of tubulin in U2OS cells. The cells were fixed with 4% formaldehyde for 20 mins, permeabilized with 0.5% Triton X-100 in PBS for 20 mins, washed 3X in PBS and blocked with 3% BSA in PBS for 30 mins at RT. Cells were stained with a tubulin antibody at a dilution of 1:1000 in 3% BSA in PBS for 1 hr at RT, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 594 goat anti-rabbit IgG secondary antibody (Product # A32740) prepared in 3% BSA in PBS at a dilution of 1:1000 for 1 hr at RT in the presence of NucBlue Live ReadyProbes Reagent (Product # R37605). The image contains overlay of tubulin (red) and nuclei (blue). Images were taken on an EVOS FL Auto 2 Imaging System (Product # AMAFD2000) with an Olympus 20X Super Apochromat objective (Product # AMEP4754) at 40X magnification. Actin was stained using Alexa Fluor Plus Phalloidin (Product # A30105)

View more figures on thermofisher.cn

332 References

Engineering aortic valves via transdifferentiating fibroblasts into valvular endothelial cells without using viruses or iPS cells. Bioact Mater (2025)

Pyroptotic cell corpses are crowned with F-actin-rich filopodia that engage CLEC9A signaling in incoming dendritic cells. Nat Immunol (2025)

Corneal strain influences keratocyte proliferation and migration through upregulation of ALDH3A1 expression. FASEB J (2024)

Non-cell autonomous regulation of cell-cell signaling and differentiation by mitochondrial ROS. J Cell Biol (2024)

EPRS1-mediated fibroblast activation and mitochondrial dysfunction promote kidney fibrosis. Exp Mol Med (2024)