Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 405

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 405
Excitation/Emission Max	404/455 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_2890547

Applications	Tested Dilution	Publications
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-

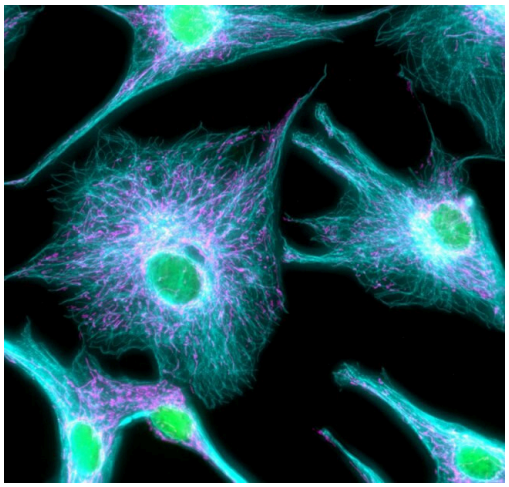
Product Specific Information

To minimize cross-reactivity, the donkey anti-rabbit IgG whole antibodies have been cross-adsorbed against serum proteins 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.

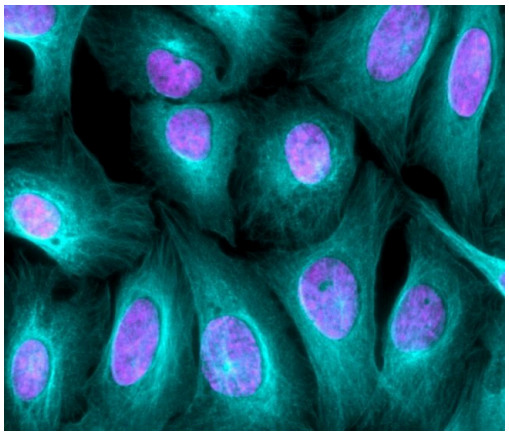
Specificity: This antibody binds to heavy chains on rabbit IgG and light chains on all rabbit immunoglobulins. This antibody does not bind non-immunoglobulin rabbit serum proteins or IgG from bovine, goat, chicken, guinea pig, hamster, horse, sheep, mouse, rat, or human.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 405



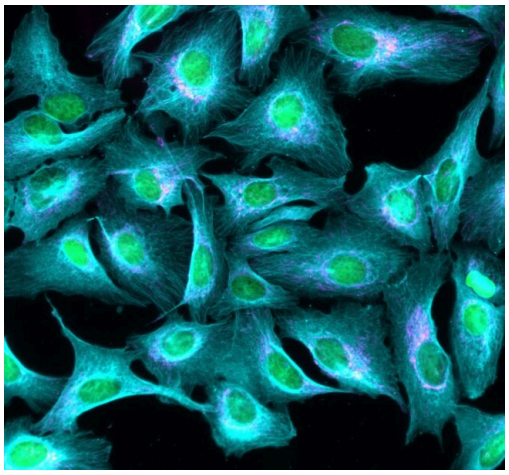
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A48258) in ICC/IF

Immunofluorescent analysis of tubulin in BPAE cells. The cells were fixed with 4% fromaldehyde for 20 mins, washed 3x in PBS, permeablized with 0.5% Triton X-100 in PBS for 20 minutes, washed 3x in PBS and blocked with 3% BSA in PBS for 30 minutes. Cells were incubated with mouse anti-ATP Synthase and rabbit anti-tubulin diluted in 3% BSA in PBS overnight at 4C and washed 3x in PBS. Cells were then incubated with Alexa Fluor Plus 405 Donkey anti-rabbit at a dilution of 1:250 and Alexa Fluor Plus 647 Donkey anti-mouse (Product # A32787) at a dilution of 1:500 in the presence of 3% BSA in PBS and Sytox Green (Product # S7020) at a dilution of 1:10k for 1 hour at room temperature followed by 3 washes in PBS. The images contain overlay of mitochondria (magenta), tubulin (cyan) and nuclei (green). Images were taken on an EVOS M7000 Imaging system (Product # AMF7000) at 40X magnification.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A48258) in ICC/IF

Immunofluorescent analysis of tubulin in HeLa cells. The cells were fixed with 4% fromaldehyde for 20 mins, washed 3x in PBS, permeablized with 0.5% Triton X-100 in PBS for 20 minutes, washed 3x in PBS and blocked with 3% BSA in PBS for 30 minutes. Cells were incubated with rabbit anti-tubulin diluted in 3% BSA in PBS overnight at 4C and washed 3x in PBS. Cells were then incubated with Alexa Fluor Plus 405 Donkey anti-rabbit prepared in 3% BSA in PBS at a dilution of 1:250 in the presence of Sytox Deep Red (Product # S11381) at a dilution of 1:20k for 1 hour at room temperature followed by 3 washes in PBS. The images contain overlay of tubulin (cyan), and nuclei (magenta). Images were taken on an EVOS M7000 Imaging system (Product # AMF7000) at 20X magnification.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A48258) in ICC/IF

Immunofluorescent analysis of tubulin in U2OS cells. The cells were fixed with 4% fromaldehyde for 20 mins, washed 3x in PBS, permeablized with 0.5% Triton X-100 in PBS for 20 minutes, washed 3x in PBS and blocked with 3% BSA in PBS for 30 minutes. Cells were incubated with rabbit anti-tubulin diluted in 3% BSA in PBS overnight at 4C and washed 3x in PBS. Cells were then incubated with Alexa Fluor Plus 405 Donkey anti-rabbit prepared in 3% BSA in PBS at a dilution of 1:250 in the presence of Sytox Deep Red (Product # S11381) at a dilution of 1:20k for 1 hour at room temperature followed by 3 washes in PBS. The images contain overlay of tubulin (cyan), and nuclei (magenta). Images were taken on an EVOS M7000 Imaging system (Product # AMF7000) at 20X magnification

14 References

hGRAD: A versatile "one-fits-all" system to acutely deplete RNA binding proteins from condensates. J Cell Biol (2024)

-H-Spectrin is a key component of an apical-medial hub of proteins during cell wedging in tube morphogenesis bioRxiv (2023)

Rho/ROCK activity tunes cell compartment segregation and differentiation in nephron-forming niches bioRxiv (2023)

The PrLGluavBNSTGABA circuit rapidly modulates depression-like behaviors in male mice. iScience (2023)

Alveolar repair following LPS-induced injury requires cell-ECM interactions. JCI Insight (2023)