

# Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488

Product Details	
Size	1 mg
Species Reactivity	Chicken
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 488
Excitation/Emission Max	493/518 nm
Immunogen	Purified Chicken IgY, Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	Affinity chromatography
Storage buffer	proprietary buffer, pH 6.5
Contains	0.016% Methylisothiazolone
Storage conditions	4° C, store in dark
RRID	AB_2762843

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

## Product Specific Information

To minimize cross-reactivity, the goat anti-chicken IgY whole antibodies have been affinity-purified and cross-adsorbed against chicken serum containing non-immunoglobulin chicken serum proteins. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. 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. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) 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.<sup>^M</sup>

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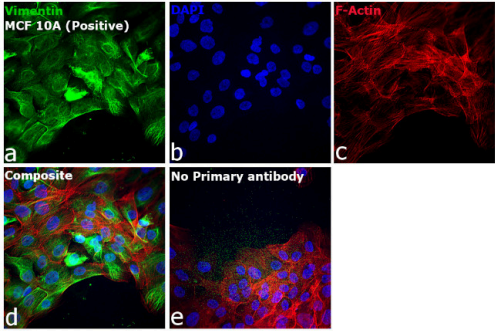
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically.<sup>^M</sup>

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Specificity: This antibody binds to heavy chains on chicken IgY and light chains on all chicken immunoglobulins. This antibody does not bind non-immunoglobulin chicken serum proteins.

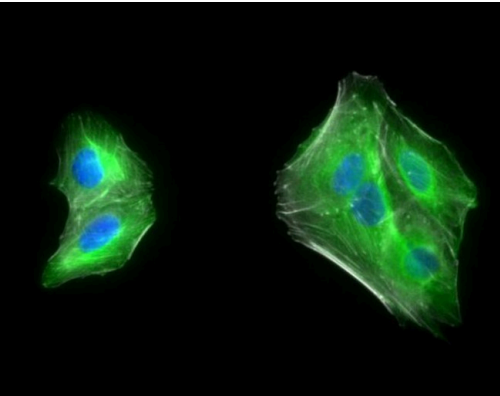
**Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32931) in ICC/IF**

Immunofluorescence analysis of Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Product # A32931) was performed using MCF10A cells stained with Vimentin Polyclonal Antibody (Product # PA1-10003). 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 primary antibody (1:200 dilution in 0.1% BSA) for 3 hours at room temperature. Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32931, 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). Alexa Fluor™ 647 Phalloidin (Product # A22287, 1:4000) (Panel c: red). Panel d represents the composite image. Nonspecific staining was not observed with secondary antibody alone (panel e). The images were captured at 60X magnification.



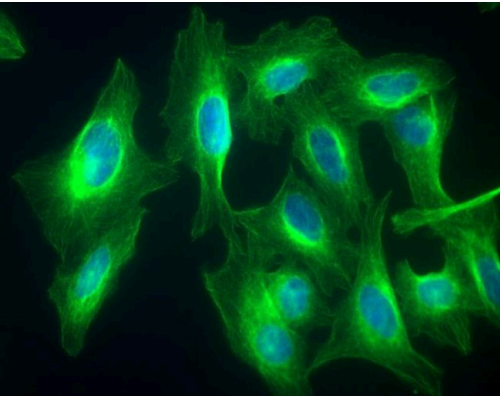
**Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32931) in ICC/IF**

Immunofluorescent analysis of tubulin in A549 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 1:500 in 3% BSA in PBS over night at 4C, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 488 goat anti-chicken IgY secondary antibody (Product # A32931) prepared in 3% BSA in PBS at a dilution of 1:500 for 1 hr at RT in the presence of NucBlue Live ReadyProbes Reagent (Product # R37605). The image contains overlay of tubulin (green) and nuclei (blue). Images were taken on an EVOS FL Auto 2 Imaging System (Product # AMAFD2000) with an Olympus 40X Super Apochromat objective (Product # AMEP4754) at 40X magnification. Actin was stained using Alexa Fluor Plus Phalloidin (Product # A30105).



**Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32931) 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 1:500 in 3% BSA in PBS over night at 4C, washed 3X in PBS and then incubated with Invitrogen Alexa Fluor Plus 488 goat anti-chicken IgY secondary antibody (Product # A32931) prepared in 3% BSA in PBS at a dilution of 1:500 for 1 hr at RT in the presence of NucBlue Live ReadyProbes Reagent (Product # R37605). The image contains overlay of tubulin (green) and nuclei (blue). Images were taken on an EVOS FL Auto 2 Imaging System (Product # AMAFD2000) with an Olympus 40X Super Apochromat objective (Product # AMEP4754) at 40X magnification.



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