

Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594

Product Details	
Size	1 mg
Species Reactivity	Chicken
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 594
Excitation/Emission Max	590/618 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, 0.016% Bromonitrodioxane
Storage conditions	4° C, store in dark
RRID	AB_2762829

Applications	Tested Dilution	Publications
Western Blot (WB)	1:2,000	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-

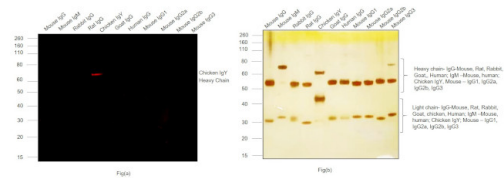
Product Specific Information

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.

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 chicken IgY and light chains on all chicken immunoglobulins. This antibody does not bind non-immunoglobulin chicken serum proteins.

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

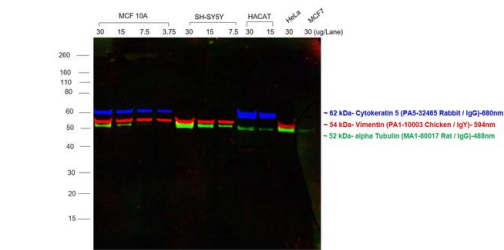


Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32759)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Chicken IgY. Protein bands at ~68 kDa corresponding to Chicken IgY Heavy Chain was observed in Chicken IgY but not in other species using Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 (Product # A32759) in Western Blot. Relative expression. {RE}

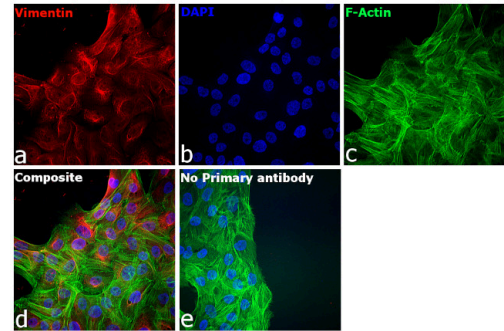
Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32759) in WB

Multiplexed fluorescent western blot was performed using Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 (Product # A32759). Whole cell extracts of MCF 10A (Lane 1, 2), HeLa (3, 4), SH-SY5Y (Lane 5, 6, 7) and MCF7 (Lane 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were transferred onto nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Vimentin Chicken IgY Polyclonal Antibody (Product # PA1-10003), Cytokeratin 5 Rabbit Polyclonal Antibody (Product # PA5-32465) and alpha Tubulin Monoclonal Antibody (YL1/2) (Product # MA1-80017). Secondary antibodies (Product # A32759), (Product # A32808) and (Product # A48269) were used for detection of Vimentin, Cytokeratin 5 and alpha tubulin respectively. Fluorescent detection was performed using iBrightFL1500 (Product # A44115). The anti-chicken secondary antibody (Product # A32759) specifically detects the chicken primary antibody and not the mouse and rabbit primary antibodies.



Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody (A32759) in ICC/IF

Immunofluorescence analysis of Goat anti-Chicken IgY (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 (Product # A32759) 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 594 (Product # A32759, 1:200 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) and Alexa Fluor™ 488 Phalloidin (Product # A12379, 1:300) (Panel c: green). Panel d represents the composite image. Nonspecific staining was not observed with secondary antibody alone (panel e). The images were captured at 60X magnification.



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26 References

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