

# Chicken anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594

Product Details	
Size	1 mg
Species Reactivity	Rat
Host/Isotype	Chicken / IgY
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 594
Excitation/Emission Max	590/618 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4°C, store in dark
RRID	AB_2535874

Applications	Tested Dilution	Publications
Western Blot (WB)	1:2,500-1:5,000	-
Immunohistochemistry (IHC)	1-10 µg/mL	0 Publication
Immunocytochemistry (ICC/IF)	1-2 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

## Product Specific Information

Chicken anti-rat antibodies react with IgG heavy chains and all classes of immunoglobulin light chains from rat. Chicken secondary antibodies have gained popularity because they demonstrate a lower level of nonspecific binding. Chicken antibodies lack a classic &quot;Fc&quot; domain and will not bind to protein A or protein G, nor will they bind to mammalian IgG Fc receptors.

Product will be shipped at Room Temperature.

Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21471)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rat IgG. Bands at ~55 and 25 kDa corresponding to Rat IgG Heavy Chain and Light Chain were observed in Rat IgG, Rat IgG2a and Rat IgG2b but not in other species using Chicken anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Product # A-21471) in Western Blot.

Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21471) in ICC/IF

Immunofluorescence analysis of Chicken anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Product # A-21471) was performed using NTERA-2 (positive model) and HeLa (negative model) in cells stained with SOX2 Monoclonal Antibody (Btjce, eBioscience (Product # 14-9811-82). 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 1:100 dilution of primary antibody overnight at 4C. Chicken anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (Product # A-21471, 1:2000 dilution) in 0.1% BSA in PBS for 1 hour at room temperature, was used for detection of SOX2 in the nucleus (Panel a: Red). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:500) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in HeLa (negative model for SOX2) 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).

Rat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21471) in ICC/IF

Immunofluorescence analysis of Chicken anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 594 conjugate was performed using A549 cells stained with alpha Tubulin (YL1/2) Rat Monoclonal Antibody (Product # MA1-80017). 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 2µg/mL Rat primary antibody for 3 hours at room temperature. Chicken anti-Rat IgG (H+L) Secondary Antibody, Alexa Fluor 594 conjugate (Product # A-21471) was used at a concentration of 2µg/mL in phosphate buffered saline containing 0.2 % BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.

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