

# Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555

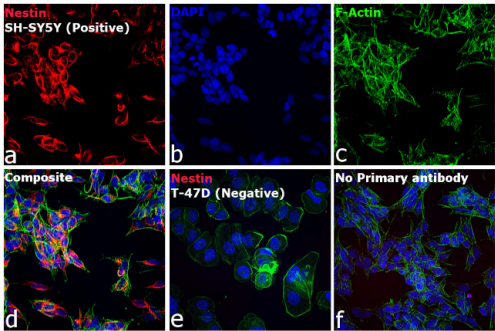
Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4°C, store in dark
RRID	AB_2535848

Applications	Tested Dilution	Publications
Western Blot (WB)	1:5000	-
Immunohistochemistry (IHC)	1-10 µg/mL	-
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

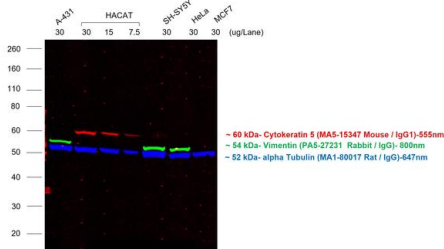
## Product Specific Information

Product will be shipped at Room Temperature.

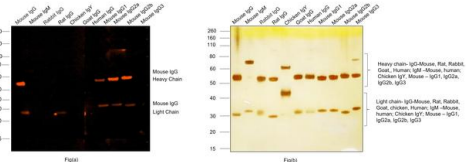
**Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21427) in ICC/IF**  
Immunofluorescence analysis of Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (Product # A-21427) was performed using SH-SY5Y (positive model) and T-47D (negative model) cells stained with Nestin Monoclonal Antibody (10C2), eBioscience™ (Product # 14-9843-80). 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. Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (Product # A21427, 1:2000 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Nestin in the cytoskeleton (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 T-47D (negative model for Nestin) 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).



**Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21427) in WB**  
Multiplexed fluorescent western blot was performed using Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (Product # A-21427). Whole cell extracts of A-431 (Lane 1), HaCaT (Lane 2, 3, 4), SH-SY5Y (Lane 5), HeLa (Lane 6) and MCF7 (Lane 7) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with Cytokeratin 5 Monoclonal Antibody (3E2F1) (Product # MA5-15347), Vimentin chicken IgY Polyclonal Antibody (Product # PA1-10003) and alpha Tubulin Monoclonal Antibody (YL1/2) (Product # MA1-80017). Secondary antibodies (Product # A-21427, 1:5000 dilution), (Product # A32935, 1:25000 diltuion) and (Product # A48269, 1:10000 dilution) were used for detection of Cytokeratin 5, Vimentin and alpha Tubulin respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # A-21427) specifically detects the mouse primary antibody and not the rabbit and the rat primary antibodies.



**Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21427) in WB**  
Western blot was performed using Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (Product # A-21427) and ~50, 25 kDa bands corresponding to Mouse IgG Heavy Chain and Light chain respectively were observed in Mouse IgG, Mouse IgG1, Mouse IgG2a, Mouse IgG2b but not in Rabbit IgG, Mouse IgM, Chicken IgY, Goat IgG, Human IgG and Mouse IgG3. Purified protein (100 ng) of Mouse IgG (Lane 1), Mouse IgM (Lane 2), Rabbit IgG (Lane 3), Rat IgG (Lane 4), Chicken IgY (Lane 5), Goat IgG (Lane 6) Human IgG (Lane 7), Mouse IgG1 (Lane 8), Mouse IgG2a (Lane 9), Mouse IgG2b and Mouse IgG3 (Fig a) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (Product # A-21427, 1:2000 dilution) and detected using theiBrightFL1500 (Product # A44115). Silver staining was performed to establish equivalent loading of purified proteins using the Pierce™ Silver Stain Kit (Product # 24612) (Fig b). This antibody also showed reactivity to Rat IgG light chain.



## 42 References

Inhibition of cathepsin L ameliorates inflammation through the A20/NF-B pathway in endotoxin-induced acute lung injury. iScience (2024)

Bioorthogonal masked acylating agents for proximity-dependent RNA labelling. Nat Chem (2024)

The essential malaria protein PfCyRPA targets glycans to invade erythrocytes. Cell Rep (2024)

Overexpression of NT-3 in the hippocampus suppresses the early phase of the adult neurogenic process. Front Neurosci (2023)

Alleviation of preeclampsia-like symptoms through PIGF and eNOS regulation by hypoxia- and NF-B-responsive miR-214-3p deletion Research Square (2023)

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