

Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 660

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 660
Excitation/Emission Max	663/691 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_2535721

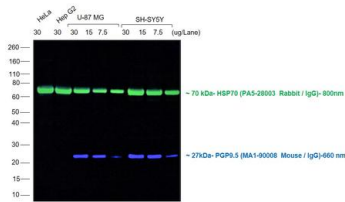
Applications	Tested Dilution	Publications
Western Blot (WB)	1:10,000	-
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

Product will be shipped at Room Temperature.

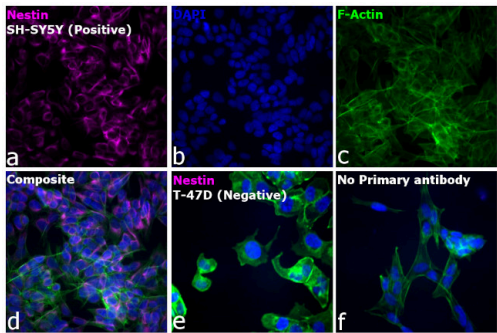
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21054) in WB

Multiplexed fluorescent western blot was performed using Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054). Whole cell extracts of HeLa (Lane 1), Hep G2 (Lane 2), U-87 MG (Lane 3, 4, 5), SH-SY5Y (Lane 6, 7, 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with PGP9.5 Monoclonal Antibody (13C4) (Product # MA1-90008) and HSP70 Polyclonal Antibody (Product # PA5-28003). Secondary antibodies (Product # A-21054, 1:10000 dilution) and (Product # A32808, 1:20000 dilution) were used for detection of PGP9.5 and HSP70 respectively. Fluorescent detection was performed using iBright FL1500 (Product # A44115). Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054) specifically detects the mouse primary antibody.



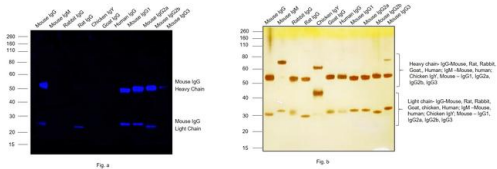
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21054) in ICC/IF

Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054) 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. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054, 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: Pink). 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-21054) in WB

Western blot was performed using Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054) and ~50, 25 kDa bands corresponding to Mouse IgG Heavy and Light Chain was observed in Mouse IgG, Mouse IgG1, Mouse IgG2a, Mouse IgG2b and Mouse IgG3 but not in Mouse IgM, Rabbit IgG, Chicken IgY, Goat IgG, Human IgG. 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) and Mouse IgG3 (Lane 10) (Fig. a) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03222BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 660 (Product # A-21054, 1:2000 dilution) and detected using the iBright FL1500 (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 towards Rat IgG light chain.



22 References

In vivo identification of Drosophila rhodopsin interaction partners by biotin proximity labeling. Sci Rep (2024)

Tissue-like environments shape functional interactions of HIV-1 with immature dendritic cells. EMBO Rep (2023)

The bacterial effector GarD shields Chlamydia trachomatis inclusions from RNF213-mediated ubiquitylation and destruction. Cell Host Microbe (2022)

Never in Mitosis Kinase 2 regulation of metabolism is required for neural differentiation bioRxiv (2022)

Comprehensive Expression Analysis of Cardiac Fibroblast Growth Factor 23 in Health and Pressure-induced Cardiac Hypertrophy. Front Cell Dev Biol (2022)

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