

Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 680
Excitation/Emission Max	681/704 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_2535728

Applications	Tested Dilution	Publications
Western Blot (WB)	1:5,000-1:50,000	0 Publication
Immunocytochemistry (ICC/IF)	1-10 µg/mL	-
Miscellaneous PubMed (Misc)	-	0 Publication

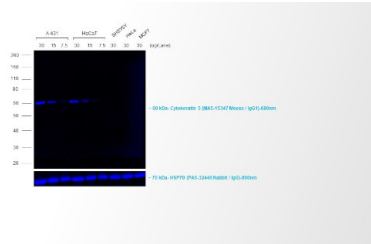
Product Specific Information

This secondary antibody is designed for fluorescent Western blot detection on various near-infrared fluorescence instruments. This antibody can be used for multi-color and multiplexing detection when using other antibodies conjugated to compatible Alexa Fluor™ dyes and wavelengths. Other applications of this antibody include immunofluorescent and fluorescent imaging applications when using instrumentation with appropriate excitation and detection capabilities.

Product will be shipped at Room Temperature.

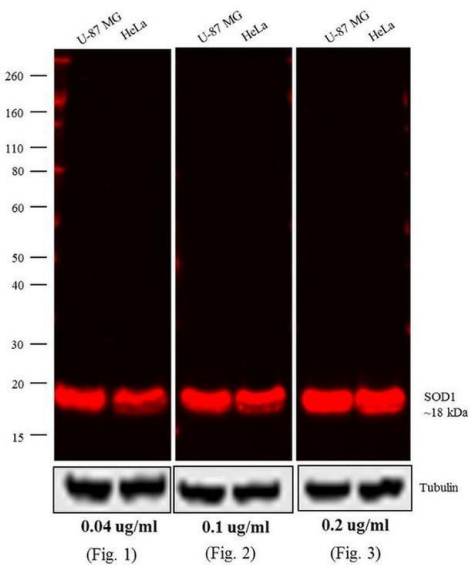
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21065) in WB

Multiplexed fluorescent western blot was performed using Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A-21065). Whole cell extracts of A-431 (Lane 1, 2, 3), HaCaT (Lane 4, 5, 6), SH-SY5Y (Lane 7), HeLa (Lane 8) and MCF7 (Lane 9) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP03221BOX). Resolved proteins were transferred onto nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Cytokeratin 5 Monoclonal Antibody (3E2F1) (Product # MA5-15347), and HSP70 Polyclonal Antibody (Product # PA5-32446). Secondary antibodies (Product # A-21065, 1:30000 dilution), and (Product # SA5-35571, 1:10000 dilution) were used for detection of Cytokeratin 5, and HSP70 respectively. Fluorescent detection was performed using iBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # A-21065) specifically detects the mouse primary antibody.



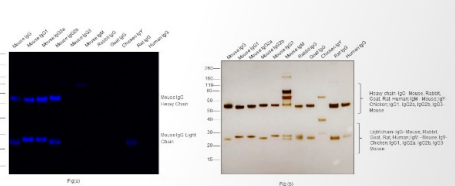
Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21065) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of U-87 MG (Lane 1) and HeLa (Lane 2). The blots were probed with Anti-SOD1 Mouse Monoclonal Antibody (Product # MA1-105, 2 µg/mL) and detected using Rabbit-anti Mouse IgG Cross Adsorbed Secondary Antibody Alexa Fluor-680 (Product # A-21065) at dilutions 0.04 µg/mL (Fig. 1), 0.1 µg/mL (Fig. 2) and 0.2 µg/mL (Fig. 3). A 18 kDa band corresponding to SOD1 was observed. Known quantity of protein samples were electrophoresed using Novex® NuPAGE®12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary antibody after blocking with 5 % skimmed milk. Fluorescent detection was performed using the Odyssey® Fc imaging system (Li-cor Biosciences).



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21065) in WB

Western blot was performed using Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A-21065) and ~55, 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 Mouse IgG3, Mouse IgM, Rabbit IgG, Goat IgG, Chicken IgY, and Human IgG. Purified protein (100 ng) of Mouse IgG (Lane 1), Mouse IgG1 (Lane 2), Mouse IgG2a (Lane 3), Mouse IgG2b (Lane 4), Mouse IgG3 (Lane 5), Mouse IgM (Lane 6), Rabbit IgG (Lane 7), Goat IgG (Lane 8), Chicken IgY (Lane 9), Rat IgG (Lane 10), Human IgG (Lane 11) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 680 (Product # A-21065, 1:5000 dilution) and detected using the iBrightFL1500 (Product # A44115). Silver staining was performed to establish equivalent loading of purified proteins using the Pierce™ Silver Stain Kit (Product # 24612) (Fig b). The antibody showed cross reactivity with Rat IgG.



9 References

Multisite phosphorylation by Cdk1 initiates delayed negative feedback to control mitotic transcription. *Curr Biol* (2022)

Early-life conditioning strategies to reduce dietary phosphorus in broilers: underlying mechanisms. *J Nutr Sci* (2020)

Glycosylation of Specific Notch EGF Repeats by O-Fut1 and Fringe Regulates Notch Signaling in *Drosophila*. *Cell Rep* (2019)

MicroRNA-124 regulates the expression of p62/p38 and promotes autophagy in the inflammatory pathogenesis of Parkinson's disease. *FASEB J* (2019)

Forms and abundance of chaperone proteins influence yeast prion variant competition. *Mol Microbiol* (2019)

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