Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ Plus 647
Excitation/Emission Max	658/675 nm
Immunogen	Gamma Immunoglobins 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_2762835

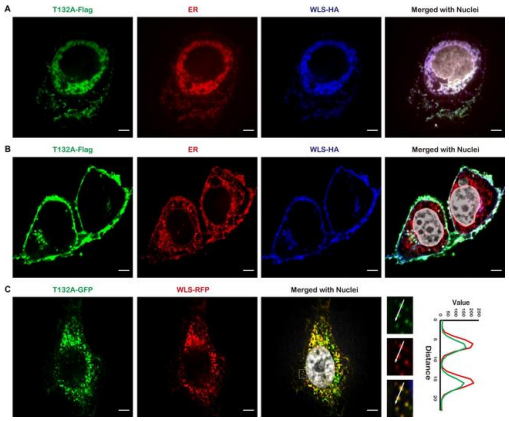
Applications	Tested Dilution	Publications
Western Blot (WB)	0.1-0.4 µg/mL	-
Immunocytochemistry (ICC/IF)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

To minimize cross-reactivity, the donkey anti-rabbit IgG whole antibodies have been cross-adsorbed against IgG from bovine, goat, chicken, guinea pig, hamster, horse, sheep, mouse, rat, and human. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in less background staining and cross-reactivity. 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. Further passages through additional columns result in highly cross-adsorbed preparations of secondary antibody. The benefits of these extra steps are apparent in multiplexing/multicolor-staining experiments 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.

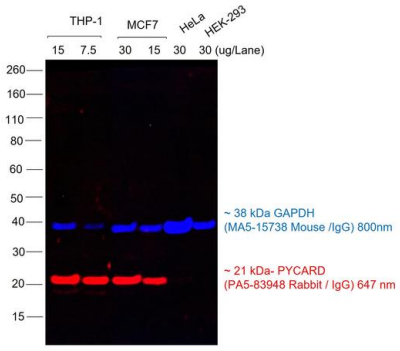
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically.

Product Images For Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647



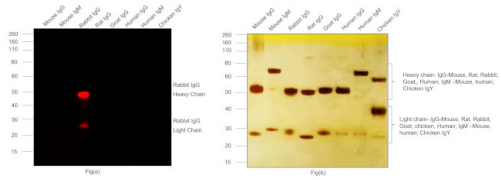
Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32795) in ICC/IF

TMEM132A colocalizes with WLS. (A,B) HeLa cells co-transfected with FL T132A-Flag, FL WLS-HA, and ER-RFP were fixed and immunostained, nuclei counterstained with Hoechst. (C) HeLa cells co-transfected with FL T132A-GFP and FL WLS-RFP were fixed and nuclei counterstained with Hoechst. The fluorescence intensity in the boxed area was quantified along the indicated white arrow for both channels. Cells were imaged under 100× oil objective lens. Fluorescent signals were pseudo-colored in green, red, blue, or gray as indicated. Scale bars: 5 µm. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33324648), licensed under a CC BY license.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32795) in WB

Multiplexed fluorescent western blot was performed using Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647 (Product # A32795). Whole cell extracts of THP-1 (Lane 1, 2), MCF7 (Lane 3, 4), HeLa (Lane 5) and HEK-293 (Lane 6) 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 PYCARD Polyclonal Antibody (Product # PA5-83948) and GAPDH Loading Control Monoclonal Antibody (GA1R) (Product # MA5-15738). Secondary antibodies (Product # A32795, 1:10000 dilution) and (Product # A32789, 1:20000 dilution) were used for detection of PYCARD and GAPDH respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-rabbit secondary antibody (Product # A32795) specifically detects the rabbit primary antibody.



Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (A32795)

Specificity of secondary antibody was demonstrated by specific detection of the target immunoglobulin. Antibody specificity was demonstrated by specific detection of Rabbit IgG. Bands at ~50 and 25 kDa corresponding to Rabbit IgG Heavy and Light Chain were observed in Rabbit IgG but not in other species using Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647 (Product # A32795) in Western Blot.Relative expression. {RE}

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184 References

A safety screening platform for individualized cardiotoxicity assessment. iScience (2024)

FOXO1-mediated lipid metabolism maintains mammalian embryos in dormancy. Nat Cell Biol (2024)

Digital automation of transdermal drug delivery with high spatiotemporal resolution. Nat Commun (2024)

Evaluating the efficacy of purchased antisense oligonucleotides to reduce mouse and human tau in vivo. Front Mol Neurosci (2024)

BPG4 regulates chloroplast development and homeostasis by suppressing GLK transcription factors and involving light and brassinosteroid signaling. Nat Commun (2024)