



Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633

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Product Details	
Size	1 mg
Species Reactivity	Goat
Host/Isotype	Donkey / IgG
Class	Polyclonal
Туре	Secondary Antibody
Conjugate	Alexa Fluor™ 633
Excitation/Emission Max	631/650 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_2535739

Applications	Tested Dilution	Publications
Western Blot (WB)	1:5000-1:10,000	0 Publication
Immunohistochemistry (IHC)	1-10 μg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunocytochemistry (ICC/IF)	1-10 μg/mL	0 Publication
Flow Cytometry (Flow)	1-10 μg/mL	-
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

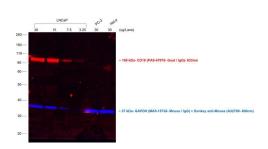
To minimize cross-reactivity, these donkey anti-goat IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against rabbit, rat, mouse, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. 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. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 633 dye is a bright, farred-fluorescent dye with excitation ideally suited to the 633 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 633 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 633 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

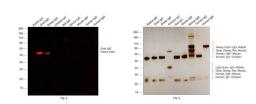
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

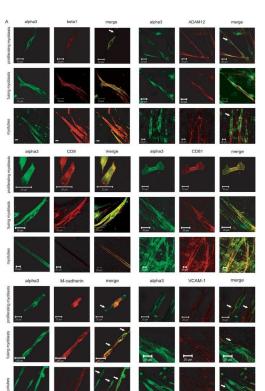
Product Images For Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21082) in WB Multiplexed fluorescent western blot was performed using Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633 (Product # A-21082). Membrane enriched extracts of LNCap (Lane 1, 2, 3, 4), PC-3 (Lane 5) and HeLa (Lane 6) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with CD10 Polyclonal Antibody (Product # PA5-47075) and GAPDH Loading Control Monoclonal Antibody (GA1R) (Product # MA5-15738). Secondary antibodies (Product # A-21082, 1: 10,000 dilution) and (Product # A32789, 1:20,000 dilution) were used for detection of CD10 and GAPDH respectively. Fluorescent detection was performed usingiBright™FL1500 (Product # A44115). The anti-goat secondary antibody (Product # A-21082) specifically detects the goat primary antibody.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21082) in WB Western blot was performed using Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor[™] 633 (Product # A-21082) and ~50 kDa band corresponding to Goat IgG Heavy Chain was observed in Goat IgG but not in Rabbit IgG, Rat IgG, Chicken IgY, Mouse IgG, Mouse IgM, Human IgG and Human IgM. Purified protein (100 ng) of Rabbit IgG (Lane 1), Goat IgG (Lane 2), Sheep IgG (Lane 3), Chicken IgY (Lane 4), Rat IgG (Lane 5), Mouse IgG (Lane 6), Mouse IgM (Lane 7), Human IgG (Lane 8), Human IgM (Lane 9) (Fig. a) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633 (Product # A-21082, 1:5000 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). The secondary antibody showed cross reactivity with Sheep IgG.



Goat IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21082) in ICC/IF Interactions beetween adhesion proteins during myoblast differentiation in vitro.A - colocalization of integrin alpha3 (green) and integrin beta1, ADAM12, CD9, CD81, M-cadherin, or VCAM-1 (red). Scale bars 50 µm. Arrows show the area where colocalization was not observed. B - Immunoprecipitation of integrin alpha3 and subsequent Western blotting analysis of CD9, CD81, M-cadherin, and V-CAM-1, c - control immunoblotting with whole cell lysate. C - interactions of analysed adhesion proteis during myoblasts differentiation. The table sumarize current study and previous results [38]. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23671573), licensed under a CC BY license.

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□ 279 References

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