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Datasheet for 610-744-002

Mouse IgG (H&L) Antibody DyLight™ 680 Conjugated

Overview

Description:	Donkey Anti-Mouse IgG (H&L) Antibody DyLight™ 680 Conjugated - 610-744-002
Item No.:	610-744-002
Size:	100 µg
Applications:	IF, WB
Reactivity:	Mouse
Host Species:	Donkey

Product Details

Background:	Anti-Mouse IgG DyLight680 Antibody generated in donkey detects reactivity to Mouse IgG. Secreted as part of the adaptive immune response by plasma B cells, immunoglobulin G constitutes 75% of serum immunoglobulins. Immunoglobulin G binds to viruses, bacteria, as well as fungi and facilitates their destruction or neutralization via agglutination (and thereby immobilizing them), activation of the compliment cascade, and opsonization for phagocytosis. The whole IgG molecule possesses both the F(c) region, recognized by high-affinity Fc receptor proteins, as well as the F(ab) region possessing the epitope-recognition site. Both the Heavy and Light chains of the antibody molecule are present. Secondary Antibodies are available in a variety of formats and conjugate types. When choosing a secondary antibody product, consideration must be given to species and immunoglobulin specificity, conjugate type, fragment and chain specificity, level of cross-reactivity, and host-species source and fragment composition.
Synonyms:	Donkey anti-Mouse IgG DyLight 680™ Conjugated Antibody, Donkey anti Mouse IgG Antibody DyLight 680™ Conjugation
Host Species:	Donkey
Specificity:	IgG (H&L)
Conjugate:	DyLight™ 680
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	2.1

Target Details

Reactivity:	Mouse
Immunogen:	Mouse IgG whole molecule
Purity/Specificity:	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Mouse IgG coupled to agarose beads followed by conjugation to fluorochrome and extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Donkey Serum, Mouse IgG and Mouse Serum. This antibody will react with heavy chains of Mouse IgG and with light chains of most Mouse immunoglobulins.

Application Details

Suggested Applications:	IF, WB (Based on references)
Application Note:	This product is designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms. The emission spectra for this DyLight™ conjugate match the principle output wavelengths of most common fluorescence instrumentation.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
FLISA:	>1:20,000
IF:	>1:5,000
WB:	>1:10,000

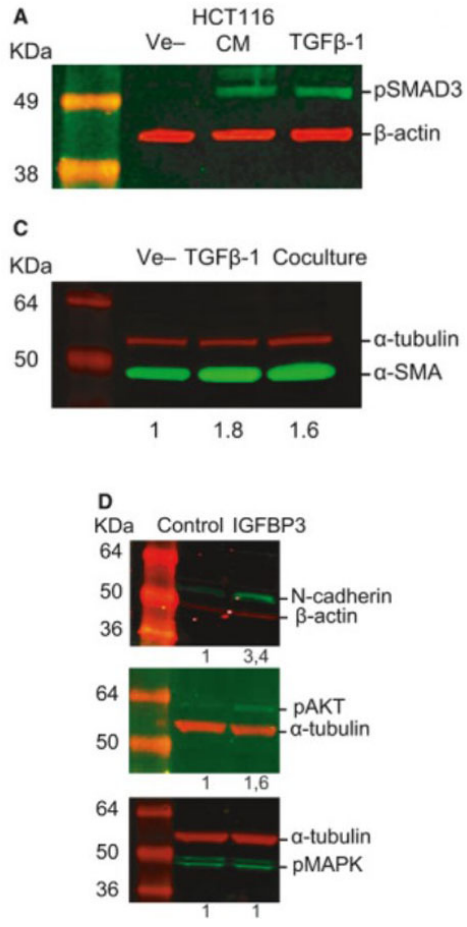
Formulation

Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

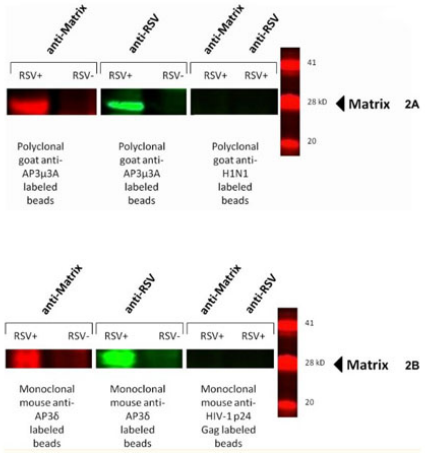
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



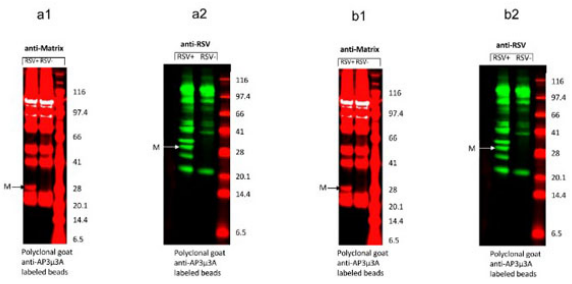
Western Blot
Western blot using Donkey Anti-Mouse IgG DyLight™680. TGF-β-mediated crosstalk between pericytes and CRC cells modulates pericyte secretome. (A) Incubation in HCT116 CM for 1 h induces SMAD3 phosphorylation in PC, as assessed by western blot. Exogenous recombinant TGF-β (10 ng·mL−1) was used as a positive control, and β-actin was used as loading control (n = 3). (C) Western blot showing increased expression of αSMA in PC cocultured with HCT116 cells or stimulated with 10 ng·mL−1 TGF-β1 for 48 h (n = 3). α-tubulin was used as loading control. Numbers indicate the expression fold change relative to the loading control. Fig. 5. PMID: 32767843.

Western Blot
Western blot using Donkey Anti-Mouse IgG DyLight™680. Insulin-like growth factor-binding protein 3 increases CRC cell migration and invasion through Akt activation. (D) Treatment with 50 ng·mL−1 IGFBP-3 for 72 h promotes the expression of N-cadherin in HCT116 cells as assessed by western blot (top panel). Phosphorylation status of Akt (middle panel) and MAPK (bottom panel) in HCT116 cells treated with 50 ng·mL−1 IGFBP-3 for 15 min. Representative images of three independent experiments (n = 3). Numbers indicate the expression fold change relative to the loading control. Fig. 7. PMID: 32767843.



Western Blot

Western blot using Donkey Anti-Mouse IgG DyLight™680. The HRSV M protein co-immunoprecipitates with the AP-3Mu3A and AP-3delta complex during HRSV infection. HEp2 cells at approximately 90% confluency were either infected at an MOI of 5 or mock infected for 24 hours, cells were scraped or proteins were subsequently extracted using MPER. Cell lysates were incubated for 6 hours with 1 µg of either polyclonal goat anti-AP-3Mu3A or monoclonal mouse anti-AP-3delta along with a antibody specific isotype control, polyclonal goat-anti H1N1or monoclonal mouse anti-HIV-1 p24 Gag at 4°C on a rotating device. 20µl Protein A/G agarose beads were added to lysate plus corresponding antibody and incubated overnight. Immunoprecipate complex was pelleted and washed with PBS and then ran out on a SDS-PAGE gel and transferred to nitrocellulose membrane. Membrane was blocked and then probed with either monoclonal mouse anti-Matrix or polyclonal goat-anti HRSV primary antibody as described previously for one hour. Membranes were then washed with a PBS-Tween20 solution extensively and then probed with species-specific secondary antibodies donkey anti-goat DyLight800 and donkey anti-mouse DyLight680. Membranes were again washed extensively and blots were imaged on Odyssey Infrared imager. The results were reproducible in at least two independent assays. Fig 2. PMID: 29028839.



Western Blot

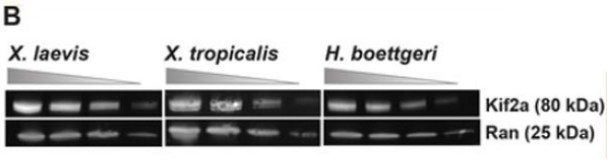
The HRSV M protein co-immunoprecipitates with the AP-3Mu3A complex during HRSV infection.
a1) The HRSV M protein co-immunoprecipitates with the AP-3Mu3A complex during HRSV infection. HEp2 cells at approximately 90% confluency were either infected at an MOI of 5 or mock infected for 24 hours, cells were scraped or proteins were subsequently extracted using MPER. Cell lysates were incubated for 6 hours with 1 µg of polyclonal goat anti-AP-3Mu3A at 4°C on a rotating device. 20µl Protein A/G agarose beads were added to lysate plus corresponding antibody and incubated overnight. Immunoprecipate complex was pelleted and washed with PBS and then ran out on a SDS-PAGE gel and transferred to nitrocellulose membrane. Membrane was blocked and then probed with monoclonal mouse anti-Matrix primary antibody as described previously for one hour. Membranes were then washed with a PBS-Tween20 solution extensively and then probed with species-specific secondary antibodies donkey

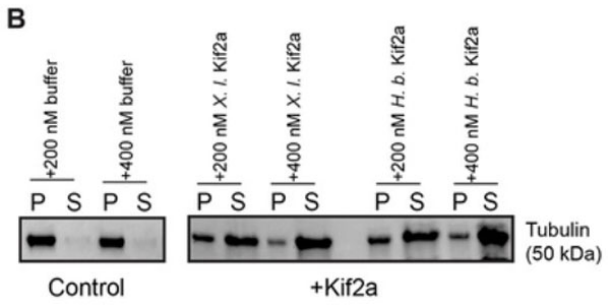
anti-mouse DyLight™680. Membranes were again washed extensively and blots were imaged on Odyssey Infrared imager. The last lane shows protein molecular weight marker (KDa). The results were reproducible in at least two independent assays. a2) The HRSV M protein co-immunoprecipitates with the AP-3Mu3A complex during HRSV infection. HEp2 cells at approximately 90% confluency were either infected at an MOI of 5 or mock infected for 24 hours, cells were scraped or proteins were subsequently extracted using MPER. Cell lysates were incubated for 6 hours with 1 µg of polyclonal goat anti-AP-3Mu3A at 4°C on a rotating device. 20µl Protein A/G agarose beads were added to lysate plus corresponding antibody and incubated overnight. Immunoprecipate complex was pelleted and washed with PBS and then ran out on a SDS-PAGE gel and transferred to nitrocellulose membrane. Membrane was blocked and then probed with polyclonal goat-anti HRSV primary antibody as described previously for one hour. Membranes were then washed with a PBS-Tween20 solution extensively and then probed with species-specific secondary antibodies donkey anti-goat DyLight™800. Membranes were again washed extensively and blots were imaged on Odyssey Infrared imager. The last lane shows protein molecular weight marker (KDa). The results were reproducible in at least two independent assays. b1) The HRSV M protein co-immunoprecipitates with the AP-3delta complex during HRSV infection. HEp2 cells at approximately 90% confluency were either infected at an MOI of 5 or mock infected for 24 hours, cells were scraped or proteins were subsequently extracted using MPER. Cell lysates were incubated for 6 hours with 1 µg of monoclonal mouse anti-AP-3delta at 4°C on a rotating device. 20µl Protein A/G agarose beads were added to lysate plus corresponding antibody and incubated overnight. Immunoprecipate complex was pelleted and washed with PBS and then ran out on a SDS-PAGE gel and transferred to nitrocellulose membrane. Membrane was blocked and then probed with monoclonal mouse anti-Matrix primary antibody as described previously for one hour. Membranes were then washed with a PBS-Tween20 solution extensively and then probed with species-specific secondary antibodies donkey anti-mouse DyLight™680. Membranes were again washed extensively and blots were imaged on Odyssey Infrared imager. The last lane shows protein molecular weight marker (KDa). The results were reproducible in at least two independent assays. b2) The HRSV M protein co-immunoprecipitates with the AP-3delta complex during HRSV infection. HEp2 cells at approximately

90% confluency were either infected at an MOI of 5 or mock infected for 24 hours, cells were scraped or proteins were subsequently extracted using MPER. Cell lysates were incubated for 6 hours with 1 µg of monoclonal mouse anti-AP-3delta at 4°C on a rotating device. 20µl Protein A/G agarose beads were added to lysate plus corresponding antibody and incubated overnight. Immunoprecipate complex was pelleted and washed with PBS and then ran out on a SDS-PAGE gel and transferred to nitrocellulose membrane. Membrane was blocked and then probed with polyclonal goat-anti HRSV primary antibody as described previously for one hour. Membranes were then washed with a PBS-Tween20 solution extensively and then probed with species-specific secondary antibodies donkey anti-goat DyLight™800. Membranes were again washed extensively and blots were imaged on Odyssey Infrared imager. The last lane shows protein molecular weight marker (KDa). The results were reproducible in at least two independent assays. Fig S1. PMID: 29028839.

Western Blot

Western blot using Donkey Anti-Mouse IgG DyLight™680. Kif2a is enriched on spindles in H. boettgeri egg extracts, and inhibition of kif2a increases spindle length.
B. Top Right Panel: Western blot of X. laevis, X. tropicalis, and H. boettgeri extracts, probed for kif2a. Bottom Panel: Quantification of 3 separate blots for each species. Band intensities were normalized to the integrated density of the corresponding Ran loading control. AU=arbitrary units.
Figure 3. PMID: 31630945.

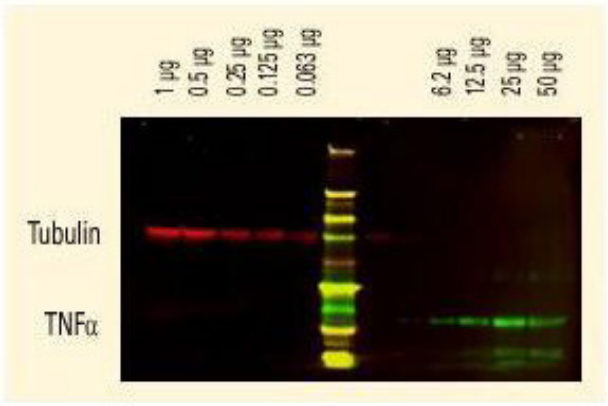




Western Blot
Serine 252 of kif2a regulates its activity.
B. Top Panel: Increasing amounts of recombinant X. laevis or H. boettgeri kif2a proteins were added to taxol-stabilized microtubules and microtubules sedimented through a sucrose cushion. Amounts of soluble tubulin in the supernatant (S) and pellet (P) were quantified by SDS-PAGE and Coomassie staining. Bottom Panel: Ratio of pellet to supernatant gel band intensities in the microtubule sedimentation assay with 200 nM H. boettgeri or X. laevis kif2a added. Bands from 3 separate gels quantified, p=0.2768. NS= Not Significant. Error bars= +/− std dev.
Figure 4. PMID: 31630945.

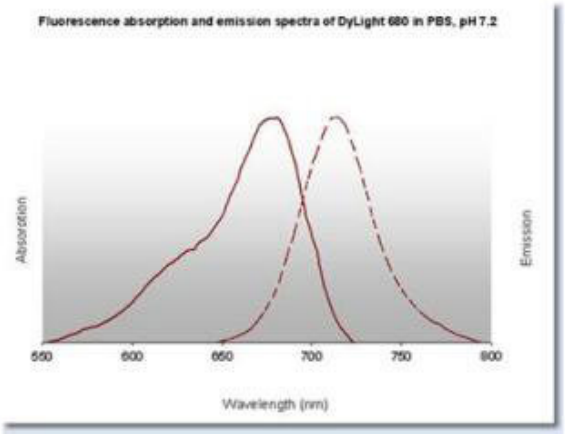
Diagram
Properties of DyLight™ Conjugates.

Emission	Color	DyLight™ Dye	Ex/Em (nm)	ε (M^{-1} cm^{-1})	Similar Dyes
Blue		**405**	**400/420**	**30,000**	**Alexa™ 405, Cascade Blue**
Green		488	493/518	70,000	Alexa™ 488, Cy2®, FITC
Yellow		549	550/568	150,000	Alexa™ 546, Alexa 555, Cy3®,TRITC
Red		649	646/674	250,000	Alexa™ 647, Cy5®
Near Infrared		680	682/715	140,000	Alexa™ 680, Cy5.5®, IRDye™ 700
Infrared		800	770/794	270,000	IRDye™ 800



Western Blot
DyLight™ dyes can be used for two-color western blot detection with low background and high signal. Anti-tubulin was detected using a DyLight™ 680 conjugate. Anti-TNFa was detected using a DyLight™ 800 conjugate. The image was captured using the Odyssey® Infrared Imaging System developed by LI-COR.

Diagram



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