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Datasheet for 611-742-127

Rabbit IgG (H&L) Antibody DyLight™ 549 Conjugated Pre-Adsorbed

Overview

Description:	Donkey Anti-Rabbit IgG (H&L) Antibody DyLight™ 549 Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) - 611-742-127
Item No.:	611-742-127
Size:	100 µg
Applications:	Dot Blot, WB, IF, IHC, Multiplex
Reactivity:	Rabbit
Host Species:	Donkey

Product Details

Background:	Anti-Rabbit IgG (H&L) DyLight 549 Antibody generated in donkey detects reactivity to Rabbit 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-Rabbit IgG Antibody DyLight 549™ Conjugated, Donkey Anti Rabbit IgG DyLight 549™ Conjugated Antibody
Host Species:	Donkey
Specificity:	IgG (H&L)
Conjugate:	DyLight™ 549
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	3.0

Target Details

Reactivity:	Rabbit
Immunogen:	Rabbit IgG whole molecule
Purity/Specificity:	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Rabbit IgG coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Donkey Serum, Rabbit IgG and Rabbit Serum. No reaction was observed against Bovine, Chicken, Goat, Guinea Pig, Hamster, Horse, Human, Mouse, Rat and Sheep Serum Proteins. This antibody will react with heavy chains of rabbit IgG and with light chains of most rabbit immunoglobulins.

Application Details

Tested Applications:	Dot Blot, WB
Suggested Applications:	IF, IHC, Multiplex (Based on references)
Application Note:	The emission spectra for this DyLight™ conjugate match the principle output wavelengths of most common fluorescence instrumentation. Anti-Rabbit IgG (H&L) DyLight 549 Antibody has been tested by dot blot and western blot and 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.
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.

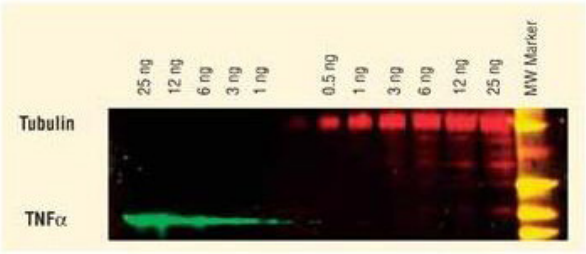
Images

Diagram
Properties of DyLight™ Conjugates.

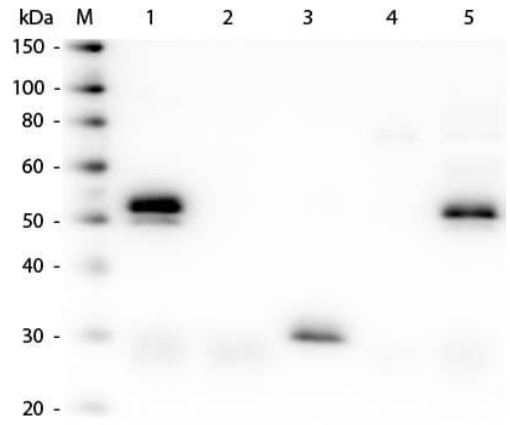
Emission	Color	DyLight™ Dye	Ex/Em (nm)	ε (M^{-1} cm^{-1})	Similar Dyes
Blue		**405**	**400/420**	**30,000**	**Alexa™ 405, Cascade Blue**
Green		488	493/518	70,000	Alexa™ 488, Cy2®, FITC
Yellow		549	550/568	150,000	Alexa™ 546, Alexa 555, Cy3®,TRITC
Red		649	646/674	250,000	Alexa™ 647, Cy5®
Near Infrared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



Western Blot
Rockland Rabbit anti-Transferrin (109-4134, lot 3033, green), Goat Anti-Alpha-1-Anti-Trypsin (100-101-147, lot 5842), and Mouse Anti-GST (200-301-200, lot 24882) were used in a multiplex system to detect target proteins under reducing (R) conditions (+4% BME) in albumin depleted human serum with 320ng of added GST. Sample was run by SDS-PAGE, transferred to 0.2 um PVDF using the BioRad Trans-Blot Turbo and blocked in 2.5% Blotto, 2.5% BSA, 0.02% Tween overnight at 4°C. Membrane was probed with three primary antibodies at 1:1000 dilution (in MB-070 overnight at 4°C). Detection shown was using DyLight549 Donkey anti-Rabbit IgG (611-742-127, lot 21100, shown as green), DyLight 488 Donkey anti-Mouse IgG (610-741-124, lot 21095, shown as blue), and DyLight 649 Donkey anti-Goat IgG (605-743-125, lot 20834, shown as red) at 1:10000 (in MB-070 30 min RT). Blots were washed, rinsed in methanol, dried and Images were collected using the BioRad VersaDoc System.



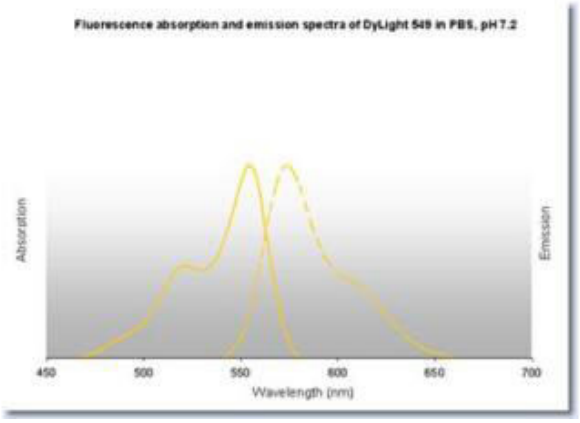
Western Blot
DyLight™ dyes can be used for two-color Western Blot detection with low background and high signal. Anti-tubulin was detected using a DyLight™ 549 conjugate. Anti-TNFa was detected using a DyLight™ 649 conjugate. The image was captured using the Typhoon™ 9410 Imaging System.



Western Blot

Western Blot of Anti-Rabbit IgG (H&L) (DONKEY) Antibody (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-701-127). Lane M: 3 µl Molecular Ladder. Lane 1: Rabbit IgG whole molecule (p/n 011-0102). Lane 2: Rabbit IgG F(ab) Fragment (p/n 011-0105). Lane 3: Rabbit IgG F(c) Fragment (p/n 010-0103). Lane 4: Rabbit IgM Whole Molecule (p/n 011-0107). Lane 5: Normal Rabbit Serum (p/n B309). All samples were reduced. Load: 50 ng of IgG, F(ab), F (c) and Serum, 25 ng of IgM. Block: MB-070 for 30 min at RT. Primary Antibody: Anti-Rabbit IgG (H&L) (DONKEY) Antibody (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-701-127) 1:7,500 for 60 min at RT. Secondary antibody: Anti-Donkey IgG (GOAT) Peroxidase Conjugated Antibody (p/n 616-1302) 1:40,000 in MB-070 for 30 min at RT. Predicted/Observed Size: 25 and 50 kDa for Rabbit IgG and Serum, 25 kDa for F(c) and F(ab), 70 and 23 kDa for IgM. Rabbit F(c) migrates slightly higher.

Diagram



References

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