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Datasheet for 610-143-002

Mouse IgG (H&L) Antibody Dylight™ 649 Conjugated

Overview

Description:	Goat Anti-Mouse IgG (H&L) Antibody DyLight™ 649 Conjugated - 610-143-002
Item No.:	610-143-002
Size:	100 µg
Applications:	Dot Blot, WB, IF, Multiplex
Reactivity:	Mouse
Host Species:	Goat

Product Details

Background:	Anti-Mouse IgG DyLight 649 Antibody generated in goat 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:	Goat Anti-Mouse IgG Secondary Antibody DyLight™649 Conjugated, Goat Anti-Mouse IgG Antibody DyLight™649 Conjugated, Anti-mouse IgG secondary antibody, anti-mouse IgG DyLight™649 conjugated secondary antibody
Host Species:	Goat
Specificity:	IgG (H&L)
Conjugate:	DyLight™ 649
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	3.2

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 followed by conjugation to fluorochrome and extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Goat 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

Tested Applications:	Dot Blot, WB
Suggested Applications:	IF, Multiplex (Based on references)
Application Note:	Anti-Mouse IgG DyLight 649 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. 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

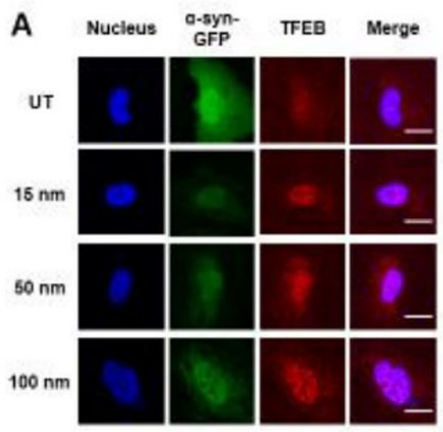
Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	100 µL

Reconstitution Buffer:	Restore with deionized water (or equivalent)

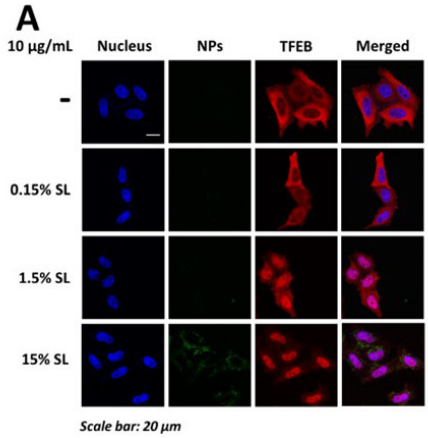
Shipping & Handling

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.

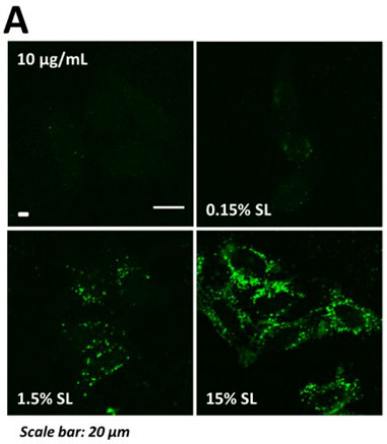
Images



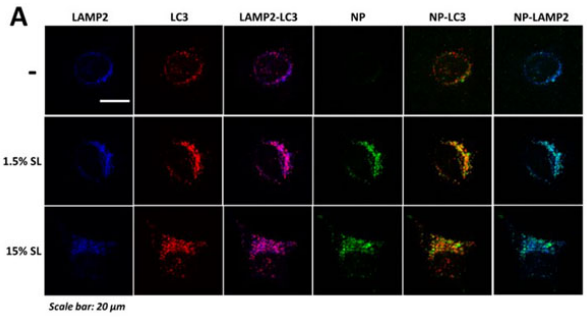
Immunofluorescence Microscopy
Accumulation of autophagic substrates in H4/ α-syn-GFP cells exposed to TiO2 NPs. (A) Confocal microscopy analyses of TFEB subcellular localization in H4/α-syn-GFP cells transfected with TFEB-3XFLAG and incubated with TiO2 NPs (100 µg/mL, 24 hrs). Representative images of colocalization of nuclei (blue, Hoechst stain, column 1) and TFEB (red, anti-TFEB, column 3) is shown in merged images (column 4). Scale bar is 20 µm. (B) Quantification of LC3-II/LC3-I levels in the soluble protein fraction of H4/α-syn-GFP cells treated with TiO2 NPs (100 µg/mL; 24 or 72 hrs) measured by Western blot. Data presented as mean ± s.d. *p<0.01. Figure 6. PMID: 30134208.



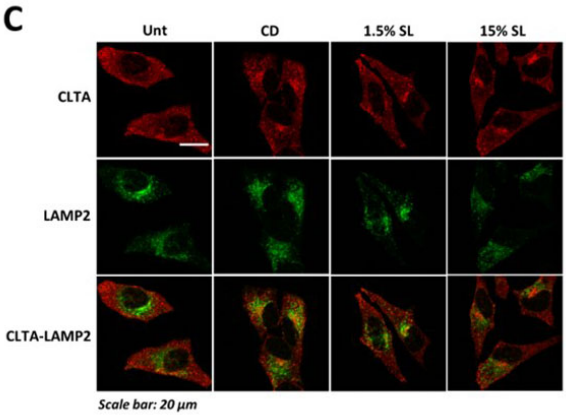
Immunofluorescence Microscopy
TFEB activation in HeLa/TFEB cells exposed to CPS-SL NPs and SL peptide. A) Confocal microscopy analysis of TFEB nuclear localization in HeLa/TFEB cells treated with CPS and CPS-SL NPs. Representative images show colocalization of the nucleus (blue, Hoechst stain, column 1) and TFEB (red, anti-FLAG, column 2) in merged images (column 3). B) Average fraction of HeLa/TFEB cells untreated and treated with 2-hydroxypropyl-β-cyclodextrin (CD, 5 mM) and SL peptide (1.5 and 7.5 µg/mL; 24 h) that present nuclear localization of TFEB. Data reported as mean ± SD (n ≥ 3). C) Average fraction of TFEB that localizes to the nucleus of HeLa/TFEB cells treated as in B. Data reported as mean ± SD (n ≥ 3). Figure S3. PMID: 31268689.



Immunofluorescence Microscopy
Uptake of CPS-SL and CPS-SA NPs in HeLa/TFEB cells, HeLa cells and fibroblasts. A) Representative confocal microscopy images of nanoparticle internalization in HeLa/TFEB cells treated with fluorescent CPS NPs and CPS-SL NPs. BC) Average fluorescence of B) HeLa/TFEB and BC) HeLa cells treated with fluorescent CPS-SA NPs containing 1.5% SA (B) 10 and 50 µg/mL; C) normalized treatment concentration; 24 h) normalized to the average fluorescence of cells treated with B) 10 µg/mL or C) 50 µg/mL CPS NPs. Data reported as mean ± SD (n ≥ 3). D) Average fluorescence of fibroblasts treated with fluorescent CPS NPs and CPS-SL NPs (at concentrations that result in comparable uptake) normalized to the average fluorescence of cells treated with 50 µg/mL CPS NPs. Data reported as mean ± SD (n ≥ 3). Figure S5. PMID: 31268689.



Immunofluorescence Microscopy
Co-localization of LC3, LAMP2, and nanoparticle aggregates, analysis of autolysosome formation, and co-localization of CLTA and LAMP2 in HeLa cells exposed to CPS-SL NPs and SL peptide. A) Confocal microscopy analyses of LC3 puncta, LAMP-LC3 colocalization, nanoparticle aggregates, NP-LC3 colocalization, and NP-LAMP colocalization in HeLa cells treated with fluorescent CPS NPs and CPS-SL NPs (normalized treatment concentrations; 24 h). Representative images show LAMP (blue, anti-LAMP-2, column 1), LC3 puncta (red, anti-LC3, column 2), the colocalization of LAMP with LC3 (column 4, merged images), intracellular nanoparticle aggregates (green, fluorescent NPs, column 4), the colocalization of nanoparticle aggregates with LC3 (column 5, merged images), and the colocalization of nanoparticle aggregates with and LAMP (column 6, merged images). B) Average number of LC3 puncta per cell and colocalization of LAMP2 with LC3 in HeLa cells, untreated, and treated with 2-hydroxypropyl-β-cyclodextrin (CD, 5 mM), and SL peptide (1.5 and 7.5 µg/mL; 24 h) C) Confocal microscopy analyses of CLTA-LAMP colocalization in HeLa cells treated with cyclodextrin (CD; 5mM; 24h) and fluorescent CPS NPs and CPS-SL NPs (normalized treatment concentrations; 24 h). Representative images show CLTA (red, anti-CLTA, row 1), LAMP (green, anti-LAMP2, row 2), and the colocalization of CLTA with LAMP (row 3, merged images). Figure S7. PMID: 31268689.



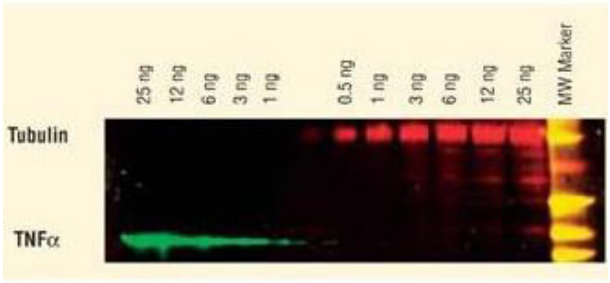
Immunofluorescence Microscopy

Co-localization of LC3, LAMP2, and nanoparticle aggregates, analysis of autolysosome formation, and co-localization of CLTA and LAMP2 in HeLa cells exposed to CPS-SL NPs and SL peptide. A) Confocal microscopy analyses of LC3 puncta, LAMP-LC3 colocalization, nanoparticle aggregates, NP-LC3 colocalization, and NP-LAMP colocalization in HeLa cells treated with fluorescent CPS NPs and CPS-SL NPs (normalized treatment concentrations; 24 h). Representative images show LAMP (blue, anti-LAMP-2, column 1), LC3 puncta (red, anti-LC3, column 2), the colocalization of LAMP with LC3 (column 4, merged images), intracellular nanoparticle aggregates (green, fluorescent NPs, column 4), the colocalization of nanoparticle aggregates with LC3 (column 5, merged images), and the colocalization of nanoparticle aggregates with and LAMP (column 6, merged images). B) Average number of LC3 puncta per cell and colocalization of LAMP2 with LC3 in HeLa cells, untreated, and treated with 2-hydroxypropyl-β-cyclodextrin (CD, 5 mM), and SL peptide (1.5 and 7.5 µg/mL; 24 h) C) Confocal microscopy analyses of CLTA-LAMP colocalization in HeLa cells treated with cyclodextrin (CD; 5mM; 24h) and fluorescent CPS NPs and CPS-SL NPs (normalized treatment concentrations; 24 h). Representative images show CLTA (red, anti-CLTA, row 1), LAMP (green, anti-LAMP2, row 2), and the colocalization of CLTA with LAMP (row 3, merged images). Figure S7. PMID: 31268689.

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

Diagram

Properties of DyLight™ Fluorescent Dyes.



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.

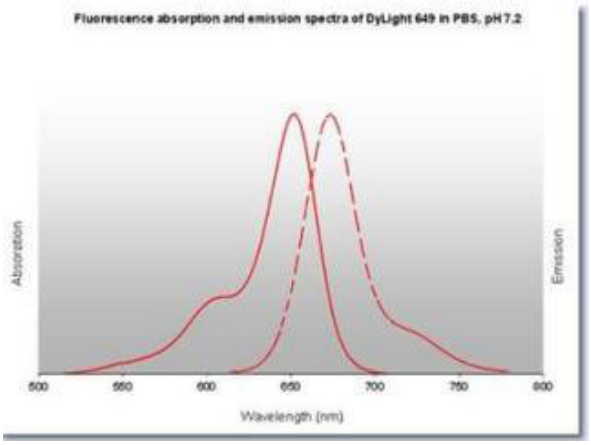


Diagram
DyLight™ 649 Fluorescence Spectra.

References

- Sun J et al. ANKRD49 promotes the metastasis of NSCLC via activating JNK-ATF2/c-Jun-MMP-2/9 axis. *BMC Cancer.* (2023)
- Legaspi SM et al. Aggregation behavior of nanoparticle-peptide systems affects autophagy. *Bioconjug Chem.* (2019)
- Popp L et al. Autophagic response to cellular exposure to titanium dioxide nanoparticles. *Acta Biomater.* (2018)

Disclaimer

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