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Datasheet for 610-143-002-0.5**Mouse IgG (H&L) Antibody DyLight™ 649 Conjugated****Overview**

Description:	Goat Anti-Mouse IgG (H&L) Antibody DyLight™ 649 Conjugated (5 X 100 µg) - 610-143-002-0.5
Item No.:	610-143-002-0.5
Size:	5 x 100 µg
Applications:	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 complement 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 Goat IgG and with light chains of most Goat immunoglobulins.

Application Details

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

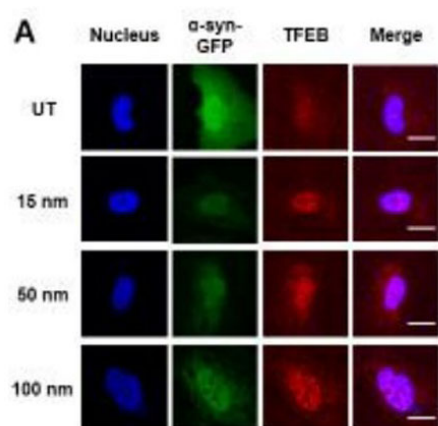
Formulation

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

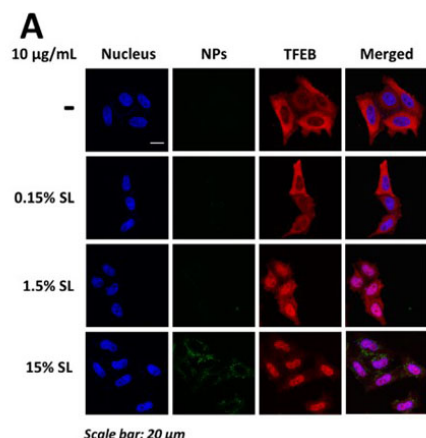
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for 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



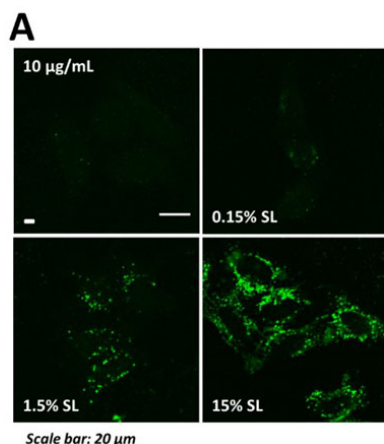
Immunofluorescence Microscopy

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 μ g/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 μ m. (B) Quantification of LC3-II/LC3-I levels in the soluble protein fraction of H4/ α -syn-GFP cells treated with TiO₂ NPs (100 μ g/mL; 24 or 72 hrs) measured by Western blot. Data presented as mean \pm s.d. * p <0.01. Figure 6. PMID: 30134208.



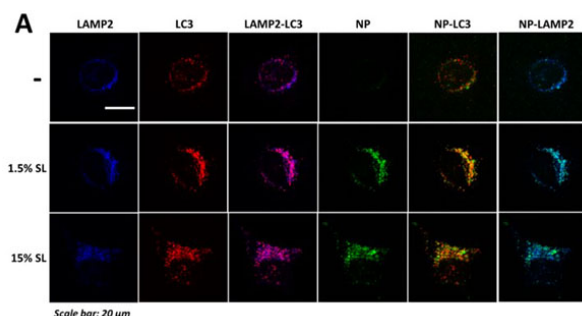
Immunofluorescence Microscopy

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 μ g/mL; 24 h) that present nuclear localization of TFEB. Data reported as mean \pm SD ($n \geq 3$). C) Average fraction of TFEB that localizes to the nucleus of HeLa/TFEB cells treated as in B. Data reported as mean \pm SD ($n \geq 3$). Figure S3. PMID: 31268689.



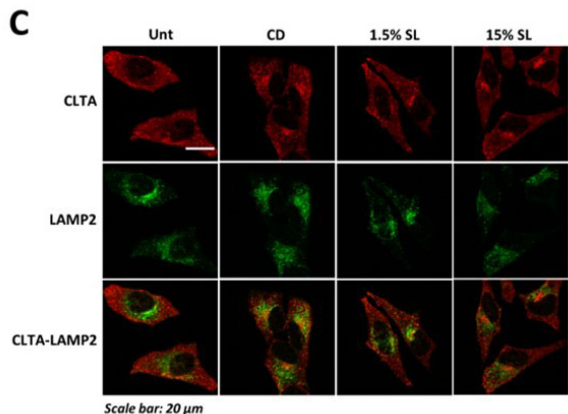
Immunofluorescence Microscopy

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 μ g/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.

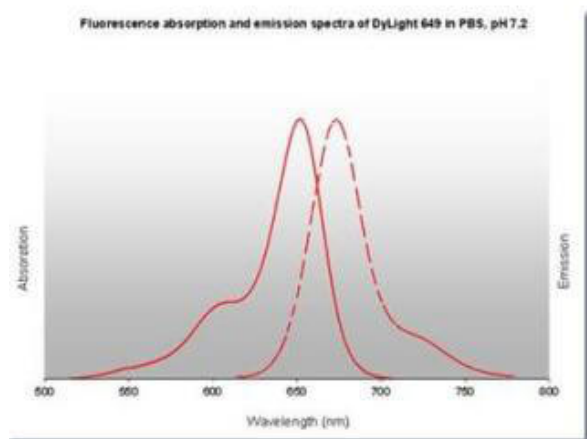
Western Blot

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-TNFα was detected using a DyLight™ 649 conjugate. The image was captured using the Typhoon™ 9410 Imaging System.



Diagram

DyLight™ 649 Fluorescence Spectra.



References

- 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

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.