

## Datasheet for 610-744-124

**Mouse IgG (H&L) Antibody DyLight™ 680 Conjugated Pre-Adsorbed****Overview**

<b>Description:</b>	Donkey Anti-Mouse IgG (H&L) Antibody DyLight™ 680 Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Rb Rt & Sh Serum Proteins) - 610-744-124
<b>Item No.:</b>	610-744-124
<b>Size:</b>	100 µg
<b>Applications:</b>	Dot Blot, ELISA, IF, WB
<b>Reactivity:</b>	Mouse
<b>Host Species:</b>	Donkey

**Product Details**

<b>Background:</b>	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.
<b>Synonyms:</b>	Donkey anti-Mouse IgG DyLight 680™ Conjugated Antibody, Donkey anti Mouse IgG Antibody DyLight 680™ Conjugation
<b>Host Species:</b>	Donkey
<b>Specificity:</b>	IgG (H&L)
<b>Conjugate:</b>	DyLight™ 680
<b>Clonality:</b>	Polyclonal
<b>Format:</b>	IgG
<b>F/P Ratio:</b>	2.0

## Target Details

<b>Reactivity:</b>	Mouse
<b>Immunogen:</b>	Mouse IgG whole molecule
<b>Purity/Specificity:</b>	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Mouse 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, Mouse IgG and Mouse Serum. No reaction was observed against Bovine, Chicken, Goat, Guinea Pig, Hamster, Horse, Human, Rabbit, Rat and Sheep Serum Proteins. This antibody will react with heavy chains of mouse IgG and with light chains of most mouse immunoglobulins.

## Application Details

<b>Tested Applications:</b>	Dot Blot, ELISA
<b>Suggested Applications:</b>	IF, WB (Based on references)
<b>Application Note:</b>	Anti-Mouse IgG DyLight680 Antibody has been tested by ELISA and dot 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.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>FLISA:</b>	>1:20,000
<b>IF:</b>	>1:5,000
<b>WB:</b>	>1:10,000

## Formulation

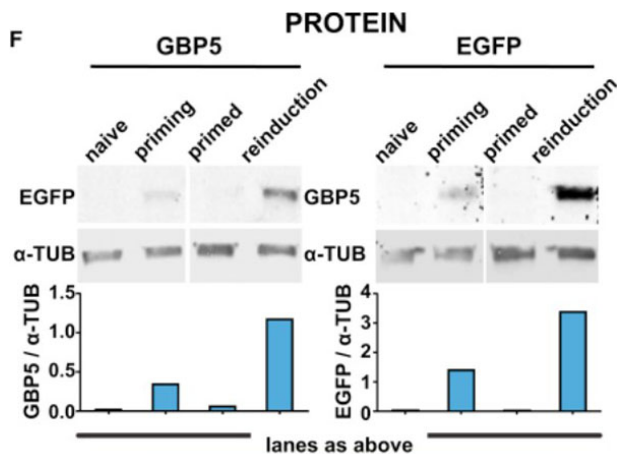
<b>Physical State:</b>	Lyophilized
<b>Concentration:</b>	1.0 mg/mL by UV absorbance at 280 nm
<b>Buffer:</b>	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
<b>Preservative:</b>	0.01% (w/v) Sodium Azide
<b>Stabilizer:</b>	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free

Reconstitution Volume:	100 $\mu$ L
Reconstitution Buffer:	Restore with deionized water (or equivalent)

## Shipping & Handling

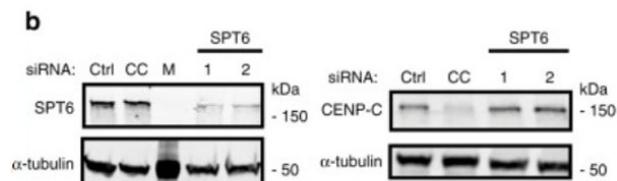
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for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



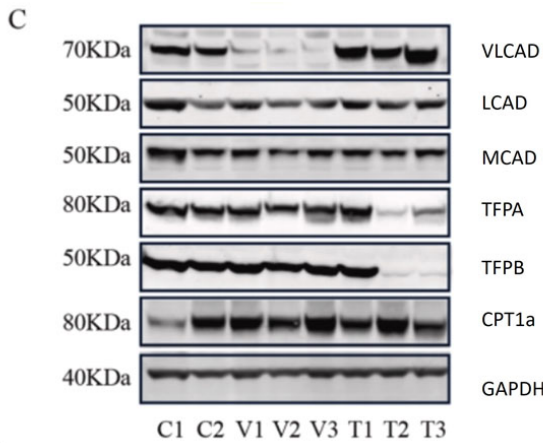
### Western Blot

Priming Results in Increased Frequency of Activation and Enhanced GBP5 Expression upon Reinduction. (F) EGFP::GBP5 cells were subjected to the IFN $\gamma$  treatment regimen outlined in Figure 1B, processed for fluorescence western blotting, and probed for GBP5 and EGFP expression.  $\alpha$ -TUB, loading control. Tubulin-normalized fluorescence intensities are plotted. Fig 3. PMID: 33108759.



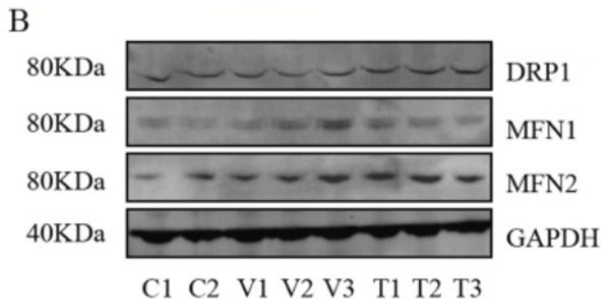
### Western Blot

Depletion of human SPT6 leads to the loss of CENP-A maintenance. HeLa cells expressing SNAP-tagged CENP-A were treated with TMR-star to detect previously incorporated CENP-A and siRNA-treated to deplete proteins indicated in (b, c). Cells were then synchronized in S phase by a thymidine block and released. Cells were allowed transit through G1 phase and were collected at the next G1/S boundary by re-addition of thymine. b Cells were treated with indicated siRNAs for 48 h and extracts were processed for immunoblotting and probed with indicated antibodies. CC CENP-C, M Marker. N = 3 independent experiments. Fig 6. PMID: 32522980.



**Western Blot**

C. Representative western blots, original blots are shown in (supplementary Fig S8-9). And densitometric quantification of relative protein levels from western blots. Data are depicted as mean  $\pm$  SD,  $n = 3$ ,  $**P < 0.01$ ,  $***P < 0.001$  and  $****P < 0.0001$  by one-way ANOVA. Intracellular transport, activation, mitochondrial transport,  $\beta$ -oxidation, carnitine shuttle, and auxiliary proteins. The primary antibodies used as follows: VLCAD 1:1000, MCAD 1:1000, LCAD 1:1000, TFPA 1:500, TFPb 1:3000, CPT1 $\alpha$  1:1000, and GAPDH 1:30,000 dilutions overnight at 4 °C. The membranes were then incubated with fluorescent conjugated secondary antibodies for 1 h; DyLight 800 conjugated goat Anti-Rabbit IgG (611-145-002), DyLight 680 conjugated goat Anti-Rabbit IgG (611-144-003), DyLight 800 conjugated goat Anti-Mouse IgG (610-145-002), and DyLight 680 conjugated donkey Anti-Mouse IgG (610-744-124). Fig 1. PMID: 33725513.









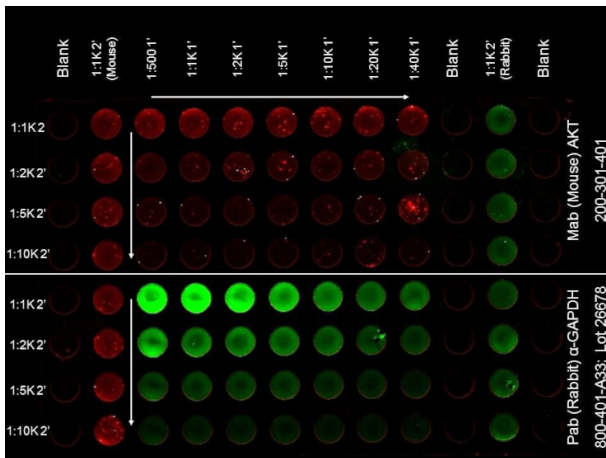
**Western Blot**

Assessment of mitochondrial fusion and fission. B. Representative western blots (original blots are shown in supplementary Fig. S10) and quantification of MFN1/2 and DRP1. No significant changes in the relative levels of proteins that facilitate mitochondrial fusion (MFN1/2) and fission (DRP1) between non-disease (control) and mutant primary fibroblasts. Data are depicted as mean  $\pm$  SD,  $n = 3$ . The primary antibodies used as follows: MFN1 1:400, MFN2 (1:400, DRP1 1:100 and GAPDH 1:30,000 dilutions overnight at 4 °C. The membranes were then incubated with fluorescent conjugated secondary antibodies for 1 h; DyLight 800 conjugated goat Anti-Rabbit IgG (611-145-002), Antibody DyLight 680 conjugated Anti-Rabbit IgG made in goat (611-144-003), DyLight 800 conjugated goat Anti-Mouse IgG (610-145-002), and DyLight 680 conjugated donkey Anti-Mouse IgG (610-744-124). Fig 3. PMID: 33725513.

### Diagram

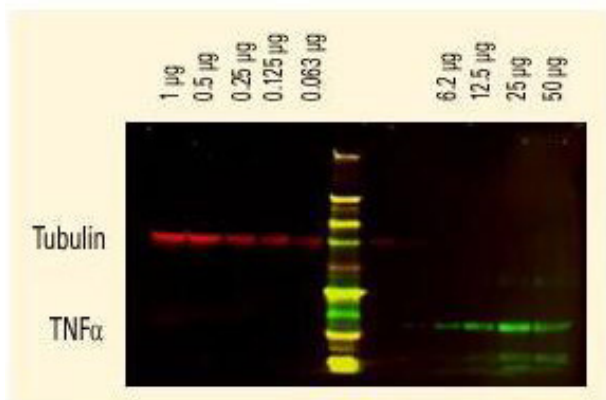
Properties of DyLight™ Conjugates.

Emission	Color	DyLight™ Dye	Ex/Em (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )	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



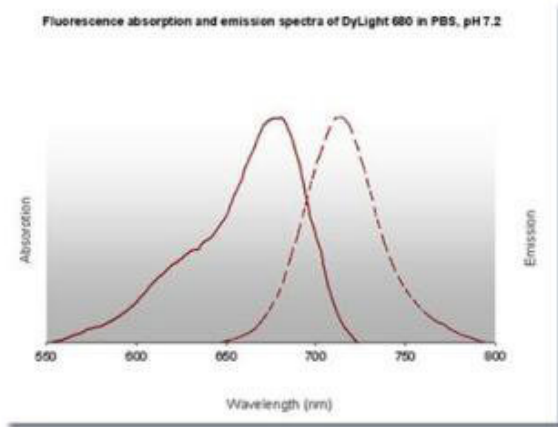
### ELISA

ELISA of DyLight™ 680 Conjugated Donkey Anti-Mouse Secondary Antibody. Antigen: HCT-116 cell line. Coating amount: Confluent in the 96 well plate. Primary antibody: AKT or GAPDH antibody at 2 µg/mL. Dilution series: Primary and Secondary Antibodies 2-fold. Mid-point concentration: N/A. Secondary antibody: DyLight™ 680 donkey secondary antibody and DyLight™ 800 goat secondary antibody starting at 1:1,000. Substrate: None.



### 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-TNFα was detected using a DyLight™ 800 conjugate. The image was captured using the Odyssey® Infrared Imaging System developed by LI-COR.

**Diagram**

## References

- Tehrani SS et al. STAT1 is required to establish but not maintain interferon- $\gamma$ -induced transcriptional memory. *EMBO J.* (2023)
- van den Berg SJW et al. p97/VCP drives turnover of SUMOylated centromeric CCAN proteins and CENP-A. *Mol Biol Cell.* (2023)
- Fritz SE et al. An alternative UPF1 isoform drives conditional remodeling of nonsense-mediated mRNA decay. *EMBO J.* (2022)
- Raimo S et al. Mitochondrial morphology, bioenergetics and proteomic responses in fatty acid oxidation disorders. *Redox Biol.* (2021)
- Bobkov GOM et al. Spt6 is a maintenance factor for centromeric CENP-A. *Nat Commun.* (2020)
- Navarro R et al. TGF- $\beta$ -induced IGFBP-3 is a key paracrine factor from activated pericytes that promotes colorectal cancer cell migration and invasion. *Mol Oncol.* (2020)
- Siwek W et al. Activation of Clustered IFN $\gamma$  Target Genes Drives Cohesin-Controlled Transcriptional Memory. *Mol Cell.* (2020)

## 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.