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Datasheet for 600-141-215

# GFP Antibody Dylight™ 488 Conjugated

## Overview

| | |
|---|---|
| **Description:** | Anti-GFP (GOAT) Antibody DyLight™ 488 Conjugated (Min X Hu Ms & Rt Serum Proteins) - 600-141-215 |
| **Item No.:** | 600-141-215 |
| **Size:** | 100 µg |
| **Applications:** | Dot Blot, WB, FC, IF, IHC, IP, Multiplex |
| **Reactivity:** | GFP, eGFP, rGFP |
| **Host Species:** | Goat |

## Product Details

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| **Background:** | GFP Dylight™ 488 Conjugated Antibody 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. |
| **Synonyms:** | goat anti-GFP Antibody DyLight™ 488 Conjugation, DyLight™ 488 conjugated goat anti-GFP antibody, Green Fluorescent Protein, GFP antibody, Green Fluorescent Protein antibody, EGFP, enhanced Green Fluorescent Protein, Aequorea victoria, Jellyfish |
| **Host Species:** | Goat |
| **Conjugate:** | DyLight™ 488 |
| **Clonality:** | Polyclonal |
| **Format:** | IgG |
| **F/P Ratio:** | 5.7 |

## Target Details

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| **Reactivity:** | GFP, eGFP, rGFP |
| **Immunogen Type:** | Recombinant Protein |
| **Immunogen:** | The immunogen is a Green Fluorescent Protein (GFP) fusion protein corresponding to the full length amino acid sequence (246aa) derived from the jellyfish Aequorea victoria. |

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| **Purity/Specificity:** | GFP Dylight™ 488 Conjugated Antibody was prepared from monospecific antiserum by immunoaffinity chromatography using Green Fluorescent Protein (Aequorea victoria) 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-Goat Serum and purified and partially purified Green Fluorescent Protein (Aequorea victoria). No reaction was observed against Human, Mouse or Rat serum proteins. |
| **Relevant Links:** | • UniProtKB - P42212 |

## Application Details

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| **Tested Applications:** | Dot Blot, WB |
| **Suggested Applications:** | FC, IF, IHC, IP, Multiplex (Based on references) |
| **Application Note:** | Anti-GFP Dylight™ 488 Conjugated Antibody has been tested by dot blot and western blot. 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. |
| **ELISA:** | 1:20,000 - 1:40,000 |
| **FLISA:** | >1:10,000 |
| **IF:** | >1:5,000 |
| **WB:** | 1:10,000 |

## Formulation

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| **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

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| **Shipping Condition:** | Ambient |

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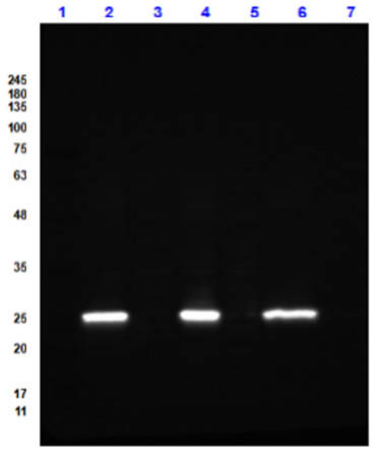
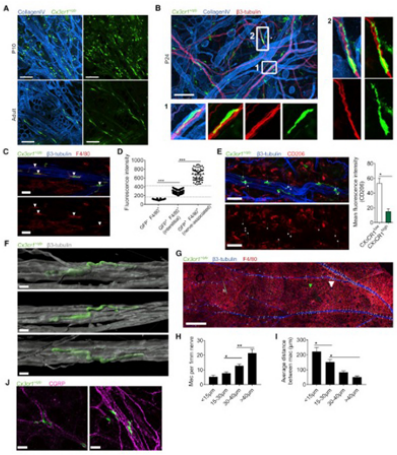
| **Storage Condition:** | Store Conjugated anti-GFP at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several 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 Bot of Goat Anti-GFP (GOAT) Antibody DyLight™ 488 Conjugated.
Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500).
Lane 2: GFP / HEK293T Whole Cell Lysate Reduced [0.05/10µg].
Lane 3: HEK293T Whole Cell Lysate Reduced (p/n W09-001-GX5) [10µg].
Lane 4: GFP / NIH-3T3 Whole Cell Lysate Reduced [0.05/10µg].
Lane 5: NIH-3T3 Whole Cell Lysate Reduced (p/n W10-000-358) [10µg].
Lane 6: GFP / PC-12 Whole Cell Lysate Reduced [0.05/10µg].
Lane 7: PC-12 Whole Cell Lysate Reduced (p/n W12-001-GL9) [10µg].
Secondary Antibody: Goat Anti-GFP (GOAT) Antibody DyLight™ 488 Conjugated at 1.0µg/mL overnight at 2-8°C.
Blocking Buffer: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) for 1 hour at RT.
Expect: 27kDa.



**Immunofluorescence Microscopy**

(A) Immunofluorescence (IF) stainings of CX3CR1hi Macs in ear skin of 10-day- and 8-week-old Cx3cr1+/gfp mice. Scale bars: 100 µm.

(B) Localization of CX3CR1hi Macs in relation to collagen IV + basement membranes and β3-tubulin+ nerve bundles in ear skin of a Cx3cr1+/gfp mouse (P24). Insets: direct alignment of CX3CR1hi Macs along nerves. Scale bar: 100 µm. Yellow fluorescent protein (YFP) and in rare cases (Figure 4B) the endogenous GFP signal in Cx3cr1+/gfp mice, was enhanced with anti-GFP DyLight488 antibody (Rockland).

(C) F4/80-positive staining of nerve-associated CX3CR1hi cells (arrowheads). Scale bars: 20 µm.

(D) Confocal microscopy of adult CX3CR1+/gfp mice (n = 50–60 cells per group of four mice in three independent experiments). GFP+ F4/80+ Macs co-localizing with β3-tubulin+ nerves, GFP+ F4/80+ interstitial Macs (partly Ly6C +), and other interstitial F4/80+ cells were analyzed for GFP fluorescence intensity. Dotted lines indicate thresholds for qualitative subset discrimination in microscopy. One-way ANOVA, Dunnett correction, ▯▯▯p < 0.01.

(E) IF staining and mean fluorescence intensity (MFI) in flow cytometry for CD206. Surrounding CD206+ cells were F4/80+ (see Figure S4G). Scale bars: 50 µm.

(F) 3D reconstruction of CX3CR1hi Macs and a β3-tubulin + nerve. Scale bars: 10 µm. Volume: 30 µm.

(G) Nerve with associated Macs (white arrows). The maximum intensity projection of 110 µm tissue volume includes CX3CR1+ γδ T cells (green triangle, exemplary) and hair follicles (white triangle, exemplary) of the epidermis. h, hair; black circle, air bubble. Scale bar: 500 µm.

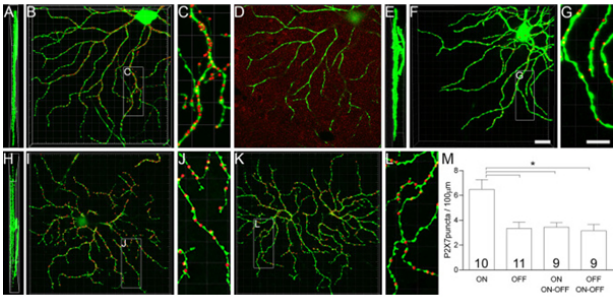
(H) Density of CX3CR1hi Macs on nerves (in Macs/mm) correlated to nerve bundle diameter (n = 4 mice).

(I) Distance between single CX3CR1hi Macs depending on nerve bundle diameter (n = 4 mice).

(J) CX3CR1hi Macs co-localized to sensory nerves (CGRP+). Scale bars: 25 µm.

Data are mean ± SEM. ▯p < 0.5, ▯▯p < 0.01 (two-tailed unpaired t test). Data are representative of at least three mice in two or more independent experiments.

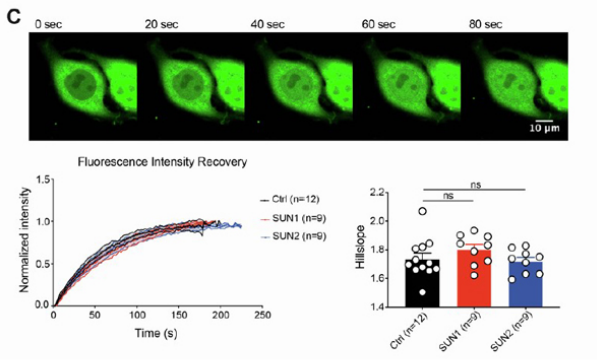
Fig 4. PMID: 31201094



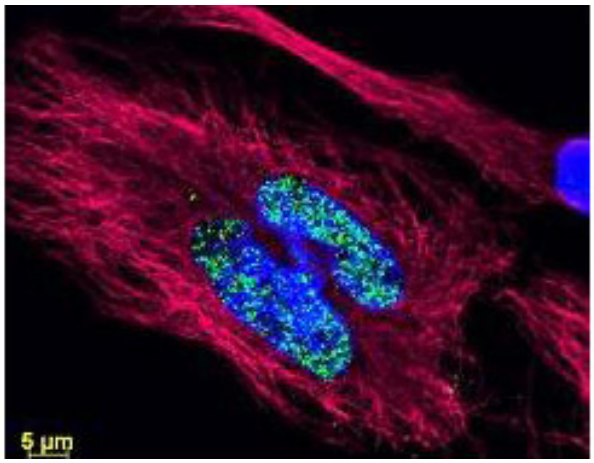

**Immunofluorescence Microscopy**

P2X7-Rs are highly expressed in ON-RGCs of Thy1-YFP-H mice. Thy1-YFP-H RGCs (green) are projections of the original confocal Z-stacks, while the P2X7-R puncta contacting the dendrites (red) are digitally rendered as spots (presented as 3 times larger than the true colocalised puncta for ease of viewing). (A–C) Thy1-YFP-H labelled ON-RGC (green) showing dense labelling for P2X7-R puncta (red) shown in (A) orthogonal view, (B) from above and (C) magnified inset from (B). (D) A single plane of raw data from the ON-cell (green) with P2X7-R staining (red). (E–G) Thy1-YFP-H labelled OFF-RGC (green) showing sparser labelling for P2X7-R puncta (red) than ON-RGCs. The OFF-RGC is shown in (E) orthogonal view, (F) from above and (G) magnified inset from F. (H–L) Thy1-YFP-H labelled ON–OFF RGC (green) in the orthogonal view (H) displaying dendritic fields stratifying in ON and OFF sublaminae. Labelling for the P2X7-R puncta (red) in the ON sublamina (I) from above and (J) magnified inset from (I). Labelling for the P2X7-R puncta (red) in the OFF sublamina (K) from above and (L) magnified inset from K. (M) Quantification of P2X7-R puncta in the ON and OFF lamina of the IPL for ON-, OFF- and ON–OFF RGCs. Numbers indicate number of cells from 4 animals. *$p < 0.01$; one-way ANOVA, Tukey's multiple comparisons test. Scale bar is 10 μm.

Fig 6. PMID: 33603067




**Immunofluorescence Microscopy**
(C) Passive diffusion from the cytoplasm to the nucleus measured by FRAP, in HeLa cells transduced with mTagBFP-2A control, SUN1 or SUN2 lentivectors and a lentivector encoding GFP. Top, representative layout of a photobleached control HeLa cell: imaging starts at t = 0 after high intensity laser exposure and ends at t = 80 seconds, when fluorescence in the nucleus has been recovered (scale bar: 5 µm). Bottom left: GFP intensity recovery over time (s). For each individual cell, the background intensity was subtracted and intensity was normalized to 0 at t0 after photobleaching while the max intensity reached during the course of each measurement was set to 1. Curves show mean and standard deviation per time point across indicated number of cells per condition. Bottom right, hillslopes for each cell were calculated via non-linear regression fit (total number of nuclei analyzed per cell line are indicated, n=1 experiment; un-paired ANOVA one-way with Dunnett's post-test, line at mean ± SEM). FS5. PMID: 34592156




**Immunofluorescence Microscopy**
Multi-color Immunofluorescence Microscopy using DyLight™ dyes. Tissue: human breast carcinoma. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: Anti-Histone and Anti-Tubulin antibody at 10 µg/mL for 1 h at RT. Secondary antibody: DyLight™ 488 conjugate and DyLight™ 549 conjugate goat secondary antibody at 1:10,000 for 45 min at RT. Localization: Histone is nuclear and Tubulin is cytoplasmic. Staining: Anti-Histone detection using a DyLight™ 488 conjugate (green) fluorescent signal and Anti-Tubulin was detected using a DyLight™ 549 conjugate (red) fluorescent signal. Nuclei were counter-stained using DAPI (blue).

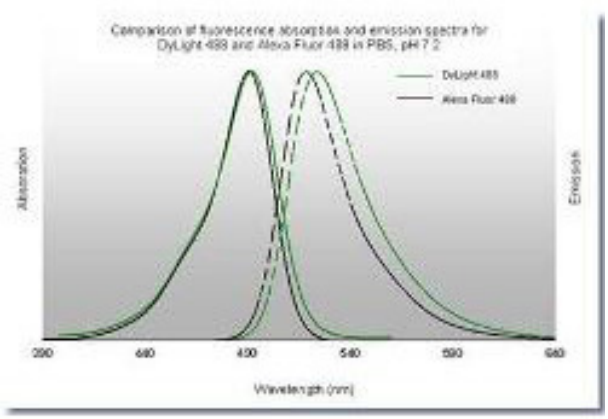
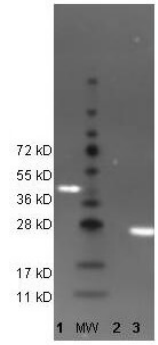



**Diagram**
Comparison of fluorescence absorbtion and emission spectra for DyLight™ 488 and Alexa Fluor 488 in PBS, pH7.2. The emission spectra for this DyLight™ conjugate match the principle output wavelengths of most common fluorescence instrumentation.

| 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. The emission spectra for this DyLight™ conjugate match the principle output wavelengths of most common fluorescence instrumentation.




**Western Blot**
Western Blot of DyLight™ 488 conjugated anti-GFP antibody to detect GFP control proteins. Lane 1: His-Sumo-GFP. Lane: Molecular Weight. Lane 2: Beta-Galactosidase (negative control). Lane 3: recombinant GFP control protein (000-001-215). Load: 35 µg per lane. Primary antibody: none. Secondary antibody: DyLight™ 488 conjugated anti-GFP goat secondary antibody at 1:5,000. Block: MB-070 for 2 hr at RT. Predicted/Observed size: 27kDa/54kDa, 27kDa for rGFP/~45kDa His-Sumo-GFP. Other band(s): none.

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