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Datasheet for 200-345-383

DYKDDDDK Tag (Anti-FLAG®) Antibody Dylight™ 800 Conjugated

Overview

Description:	Antibody for the detection of FLAG® conjugated proteins (MOUSE) Monoclonal Antibody DyLight™ 800 Conjugated - 200-345-383
Item No.:	200-345-383
Size:	100 µg
Applications:	Dot Blot, ELISA, WB
Reactivity:	FLAG-Tag
Host Species:	Mouse

Product Details

Background:	Antibody for the detection of FLAG™ recognizes FLAG™ and is optimally suited for monitoring the expression of FLAG™ tagged fusion proteins. Antibody for the detection of FLAG™ can be used to identify fusion proteins containing the FLAG™ epitope. Antibody for the detection of FLAG™ recognizes the epitope tag fused to either the amino- or carboxy- termini of targeted proteins. The epitope tag peptide sequence was first derived from the 11-amino-acid leader peptide of the gene-10 product from bacteriophage T7. DYKDDDDK is the most commonly used hydrophilic octapeptide tag.
Synonyms:	mouse anti-FLAG™ tag DyLight™800 conjugation, Enterokinase Cleavage Site (ECS), DyLight™800 conjugated mouse anti-DYKDDDDK, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
Host Species:	Mouse
Conjugate:	DyLight™ 800
Clonality:	Monoclonal
Clone ID:	29E4.G7
Format:	IgG2a
F/P Ratio:	1.6

Target Details

Reactivity:	FLAG-Tag

Immunogen Type:	Conjugated Peptide
Immunogen:	This antibody was produced in mice by repeated immunizations with a synthetic peptide corresponding to the FLAG™ epitope tag peptide DYKDDDDK (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) conjugated to KLH using maleimide.
Purity/Specificity:	This antibody is directed against the FLAG™ epitope tag and is useful in determining its presence in over expressed proteins in various assays. The antibody recognizes the FLAG™ epitope tag (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) fused to either the amino- or carboxy- termini of targeted proteins in transfected or transformed cells.

Application Details

Tested Applications:	Dot Blot, ELISA, WB
Application Note:	Anti-FLAG DyLight™800 has been tested by ELISA, dot blot, and western blot. This product 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.
ELISA:	1:10,000 - 1:25,000
FC:	User Optimized
FLISA:	>1:20,000
IF:	>1:5,000
WB:	1:10,000 - 1:25,000

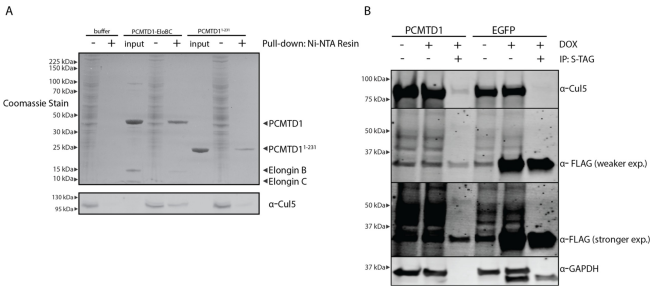
Formulation

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)

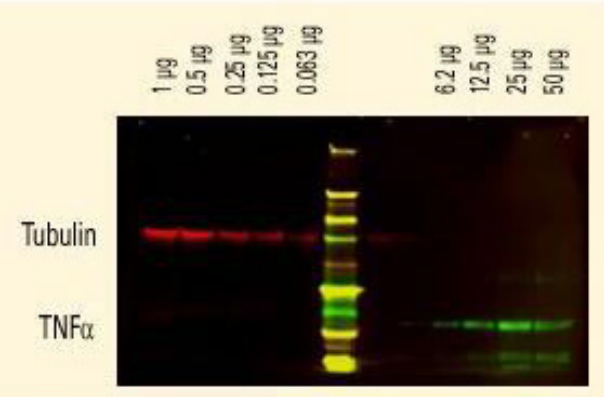
Shipping & Handling

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



Western Blot
PCMTD1 associates with Cul5 in vitro and in cells. A. PCMTD1's interaction with Cul5 was verified in vitro using pulldown assays where recombinant purified proteins were immobilized to Ni-NTA resin as prey. Input lanes represent purified protein stocks used as bait protein that was immobilized onto Ni-NTA resin for pull-down assays. Fresh RPE-1 lysates used as prey are indicated by adjacent – lanes. Proteins eluted from Ni-NTA resin are indicated by the + lanes. The bait proteins are able to co-immunoprecipitate Cul5 as shown by immunoblotting. B. PCMTD1 was also able to associate with Cul5 in cells. FLAG-S Tag-PCMTD1 or EGFP expression was induced with 0.1 µg/mL doxycycline in HeLa cells, and the resulting lysates were subjected to immunoprecipitation against the S-Tag and blotted for the indicated proteins. FLAG-S Tag-PCMTD1, but not FLAG-S Tag-EGFP, was able to co-immunoprecipitate Cul5. The blot was not stripped between the different antibodies, so the EGFP band is still present in the GAPDH image.
Fig 7. PMID: 35486881

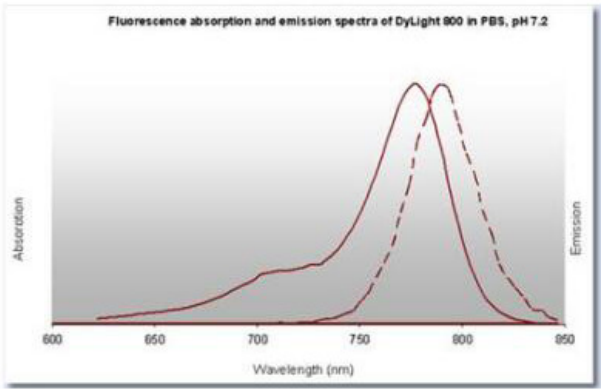


Western Blot
DyLight™ dyes can be used for two-color western blot detection with low background and high signal. Anti-tubulin was detected using a DyLight™ 680 conjugate. Anti-TNFa was detected using a DyLight™ 800 conjugate. The image was captured using the Odyssey® Infrared Imaging System developed by LI-COR.

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

Diagram



References

- Warmack RA et al. Human Protein-l-isoaspartate O-Methyltransferase Domain-Containing Protein 1 (PCMTD1) Associates with Cullin-RING Ligase Proteins. *Biochemistry.* (2022)

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