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Datasheet for 18-4516-32

Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800

Overview

Description:	Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800 - 18-4516-32
Item No.:	18-4516-32
Size:	100 µL
Applications:	Dot Blot, IP, WB
Reactivity:	Rabbit
Host Species:	Mouse

Product Details

Background:	Rabbit IgG TrueBlot® is a unique DyLight™ 800 conjugated Anti-rabbit IgG monoclonal secondary antibody. Rabbit IgG TrueBlot® enables detection of immunoblotted target protein bands, without hindrance by interfering immunoprecipitating immunoglobulin heavy and light chains. It is easy to generate publication-quality IP/Fluorescent Western Blot data with Rabbit IgG TrueBlot®, simply substitute the conventional DL800 Anti-rabbit IgG blotting reagent with Fluorescent Rabbit TrueBlot® Antibody DyLight™ 800 and follow the prescribed protocol for sample preparation and immunoblotting. Ideal for Li Cor Odyssey imaging as well as other IR and near IR imaging systems. Rabbit IgG TrueBlot® is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. TrueBlot preferentially detects the non-reduced form of rabbit IgG over the reduced, SDS-denatured form of IgG. When the immunoprecipitate is fully reduced immediately prior to SDS-gel electrophoresis, reactivity of Rabbit IgG TrueBlot® with the 55 kDa heavy chains and the 23 kDa light chains of the immunoprecipitating antibody is minimized thereby eliminating interference by the heavy and light chains of the immunoprecipitating antibody in IP/Western blot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.
Synonyms:	Anti-Rabbit IgG DL800, TrueBlot, DL800 TrueBlot ULTRA, DyLight™ 800 TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Rb TrueBlot, Infrared, IR, NIR, IR800
Host Species:	Mouse
Conjugate:	DyLight™ 800
Clonality:	Monoclonal
Clone ID:	eB182

Format:	IgG
F/P Ratio:	2.7

Target Details

Reactivity:	Rabbit
Purity/Specificity:	Fluorescent Rabbit TrueBlot® Antibody DyLight™ 800 Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography. Assay by Immunoelectrophoresis resulted in a single precipitin arc against Anti-Rabbit Serum. Reactivity is observed against native Rabbit IgG by both Western blot and ELISA.
Relevant Links:	• Fluorescent TrueBlot® Anti-Rabbit IgG DyLight™ 800 IP Western Blot Protocol • 18-4516-32 SDS • DyLight™ Antibody Spectra

Application Details

Tested Applications:	Dot Blot, IP, WB

Application Note:	Fluorescent Rabbit TrueBlot® Antibody DyLight™ 800 has been tested in dot blot, western blot, and immunoprecipitation and may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescent Rabbit TrueBlot® Antibody DyLight™ 800 is provided as a lyophilized powder. To conserve reagent, we recommend incubating the blots from minigels in sealed bags (removing as much air as possible) with minimal volume (2-3 mLs). If used conservatively at 2.5mLs/blot will yield enough reagent for 200 blots. Note that there are three key procedural considerations: 1. Protein A or G should not be used for the immunoprecipitation. Use of protein A or G beads with the rabbit TrueBlot will result in contaminating bands. For immunoprecipitation, Anti-rat IgG beads, or Anti-rabbit IgG beads should be used for rat or rabbit immunoprecipitating antibodies, respectively. 2. Immunoprecipitate should be completely reduced. 3. Bovine Serum Albumin, or MB-070 Blocking Buffer for Fluorescent Western Blotting, at low concentrations, should be used as the blocking protein for the immunoblot. DO NOT USE BLOTTO or MILK. All recommended dilutions for listed applications are intended as an initial recommendation, specific conditions for each protein and antibody combination should be specifically optimized by the end user. Fluorescence technology is widely used to detect proteins. However, many common visible fluorophores often result in considerable background fluorescence in the visible range. Visible fluorophores are rarely used for membrane-based protein detection because of this high background. DyLight™ 800 and DyLight™ 680 antibody and reagent conjugates are specifically designed for protein detection methods that use longer-wavelength, near-infrared (IR) fluorophores to visualize proteins in western blotting and other applications. Very low background fluorescence in the IR range provides for a much higher signal-to-noise ratio than visible fluorophores. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. DyLight™ 800 conjugates are optimized for the Odyssey® Infrared Imaging System developed by LI-COR. DyLight™ 800 conjugates are also suitable for immunofluorescence microscopy using commercially available excitation/emission filters in the 780nm/820nm range. Dual simultaneous labeling in western blots or microscopy is achieved when DyLight™ 800 conjugates are used in conjunction with DyLight™ 680 conjugates. DyLight™ 800 and DyLight™ 680 conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
FC:	1:2,000 - 1:10,000
FLISA:	User Optimized
IF:	1:500 - 1:2,500
IHC:	User Optimized
WB:	1:1000

Formulation

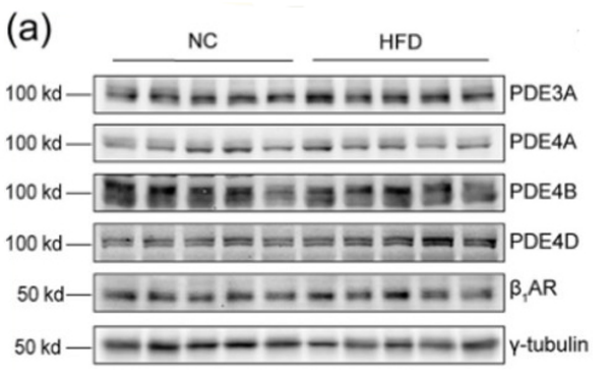
Physical State:	Lyophilized

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 Polyethylene Glycol (PEG-8000)
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

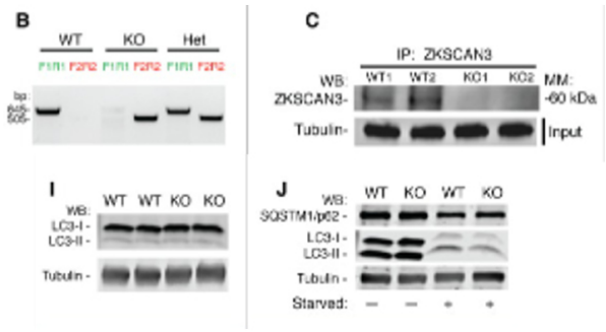
Shipping & Handling

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.

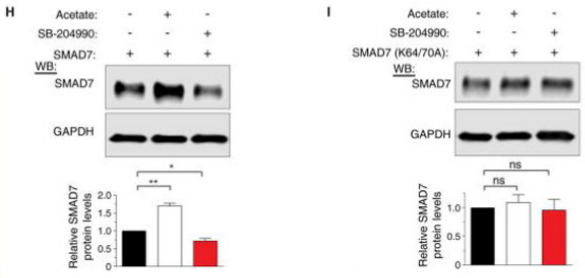
Images



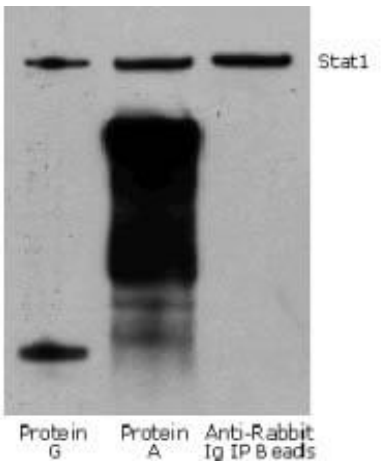
Western Blot
(a. WT mice were fed with a HFD or normal chow (NC) diet for 4.5 months. The heart tissues were harvested for western blot analysis to detect the expression of β1 -adrenoceptor, PDE3A, 4A, 4B, 4D and γ-tubulin. Fluorescent TrueBlot®: Anti-Mouse IgG DyLight™ 680 (p/n 18-4417-32) and Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800 (p/n 18-4516-32) secondary antibodies were used for multi-color detection. Fig 1. PMID: 39662482.



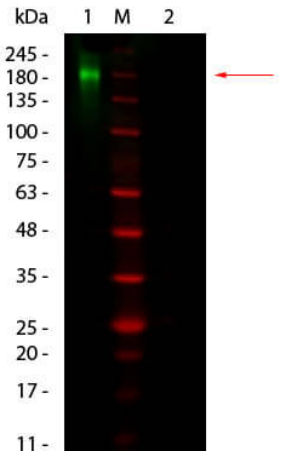
Western Blot
(B) Genotyping result demonstrates the product of primer F1 and R1 (645 bp in WT alleles) and that of F2 and R2 (505 bp in KO alleles). Heterozygous mice have both alleles. (C) Representative result of immunoprecipitation (IP) followed by western blot (WB) demonstrating loss of the 63 kD ZKSCAN3 protein in KO heart tissue. Analysis is from 2 WT (WT1, WT2) and 2 knockout (KO1, KO2) mice. Loading control for tubulin expression represents a WB of the input lysates used for immunoprecipitation. (I) Representative western blot of brain lysate of 2 WT and 2 zkscan3−/− mice demonstrating no difference in LC3B expression. (J) Representative western blot of SQSTM1/p62 and LC3 from WT and KO MEFs under fed (-) and starved (+) conditions.
Fig 1. PMID: 28581889



Western Blot
Western blot analysis using a primary rabbit polyclonal antibody to acetylated-lysine residues followed by a secondary Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800 antibody (p/n 18-4516-32).
(H and I) Western blot analysis of wildtype SMAD7 protein levels with or without acetate or SB204990 (H) or expression of the K64/70A acetylation-defective SMAD7 mutant under these same conditions (I) (n=3 independent experiments). Data represents mean ± SEM, with significance determined by one-way ANOVA with Bonferroni's multiple comparison test (A, D, E, H, I). * $p < 0.05$; ** $p < 0.01$; ns, not significant.
Fig 3. PMID: 29429925



Western Blot
Rabbit TrueBlot® IP / Western Blot: Jurkat cell lysate (0.5 ml of 1x10e7 cells/ml) was incubated with rabbit anti-human Stat1 and immunoprecipitated using Protein G, Protein A and Anti-Rabbit Ig IP Beads. Precipitate from 5x10e5 cells was subjected to electrophoresis, transferred to a PVDF membrane, and Western blotted with anti-Stat1 using Rabbit TrueBlot®: Anti-Rabbit IgG HRP



Western Blot
Western Blot of Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800. Lane 1: Rabbit IgG, Non-reduced. M: Opal Pre-stained Ladder (p/n MB-210-0500). Lane 2: Rabbit IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800 at 1:1,000 for 60 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: ~160 kDa for Rabbit IgG, Non-reduced.

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