Datasheet for ABIN6698840

Fluorescent TrueBlot®: Anti-Rabbit IgG DyLight™ 800

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Overview

Quantity:	100 µL
Target:	IgG
Reactivity:	Rabbit
Host:	Mouse
Conjugate:	DyLight 800
Application:	Flow Cytometry (FACS), Immunohistochemistry (IHC), Western Blotting (WB), FLISA, Fluorescence Microscopy (FM)

Product Details

Brand:	TrueBlot®
Characteristics:	Synonyms: 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 Background: Rabbit IgG TrueBlot® is a unique DyLight™ 800 conjugated Anti-rabbit IgG immunoblotting (second step) reagent. 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

Product Details

	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.
Purification:	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.
Labeling Ratio:	2.1

Target Details

Target:	IgG
Abstract:	IgG Products
Target Type:	Antibody

Application Details

Application Notes:	Immunohistochemistry Dilution: User Optimized Application Note: Fluorescent Rabbit TrueBlot® Antibody DyLight™ 800 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 2.5 mLs/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.

Application Details

	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. FLISA Dilution: User Optimized Flow Cytometry Dilution: 1:2,000 - 1:10,000 Western Blot Dilution: 1:1000 IF Microscopy Dilution: 1:500 - 1:2,500
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Reconstitution:	Reconstitution Volume: 100 µL Reconstitution Buffer: Restore with deionized water (or equivalent)
Buffer:	Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: 10 mg/mL Polyethylene Glycol (PEG-8000) 0.01 % (w/v) Sodium Azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	RT,4 °C,-20 °C

Handling

Storage Comment:	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.
Expiry Date:	12 months

Publications

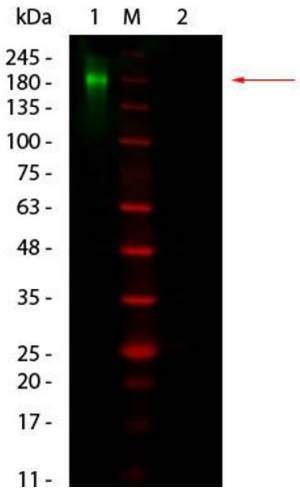
Product cited in:

Xiong, Kawagishi, Yan, Liu, Wells, Edmunds, Fergusson, Yu, Rovira, Brittain, Wolfgang, Jurczak, Fessel, Finkel: "A Metabolic Basis for Endothelial-to-Mesenchymal Transition." in: **Molecular cell** , Vol. 69, Issue 4, pp. 689-698.e7, (2019) (PubMed).

Tian, Li, Gao, Li, Yang, Wang: "Identification and validation of the role of matrix metalloproteinase-1 in cervical cancer." in: **International journal of oncology**, Vol. 52, Issue 4, pp. 1198-1208, (2018) (PubMed).

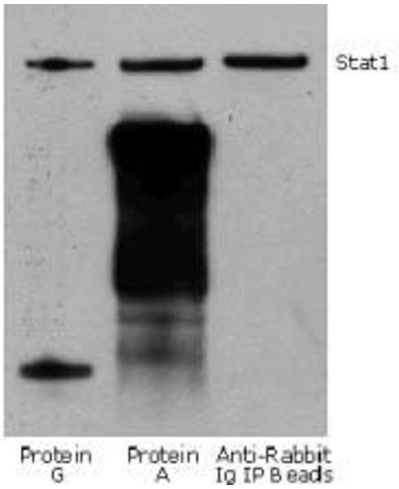
Pan, Yan, Liu, Finkel: "The role of ZKSCAN3 in the transcriptional regulation of autophagy." in: **Autophagy**, Vol. 13, Issue 7, pp. 1235-1238, (2017) (PubMed).

Images



Western Blotting

Image 1. Fluorescent: Anti-Rabbit IgG 800 Western Blot of Fluorescent: Anti-Rabbit IgG 800. Lane 1: Rabbit IgG, Non-reduced. M: Opal Pre-stained Ladder . Lane 2: Rabbit IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Fluorescent: Anti-Rabbit IgG 800 at 1:1,000 for 60 min at RT. Block: ABIN925618 for 30 min at RT. Predicted/Observed size: ~160 kDa for Rabbit IgG, Non-reduced.



Western Blotting

Image 2. Rabbit 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: Anti-Rabbit IgG HRP

