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Datasheet for 18-4517-32

Fluorescent TrueBlot®: Anti-Mouse Ig DyLight™ 800

Overview

Description:	Fluorescent TrueBlot®: Anti-Mouse Ig DyLight™ 800 - 18-4517-32
Item No.:	18-4517-32
Size:	100 µL
Applications:	IP, WB
Reactivity:	Mouse
Host Species:	Rat

Product Details

Background:	Mouse IgG TrueBlot® is a unique DyLight™ 800 conjugated Anti-mouse IgG monoclonal secondary antibody. Mouse 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 Mouse IgG TrueBlot®, simply substitute the conventional DL800 Anti-mouse IgG blotting reagent with Fluorescent Mouse TrueBlot® Antibody DyLight™ 800 and follow the prescribed protocol for sample preparation and immunoblotting. Mouse IgG TrueBlot® is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. TrueBlot preferentially detects the non-reduced form of mouse IgG over the reduced, SDS-denatured form of IgG. When the immunoprecipitate is fully reduced immediately prior to SDS-gel electrophoresis, reactivity of Mouse 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-Mouse IgG DL800, TrueBlot, DL800 TrueBlot ULTRA, DyLight™ 800 TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Ms TrueBlot, Infrared, IR, NIR
Host Species:	Rat
Conjugate:	DyLight™ 800
Clonality:	Monoclonal
Clone ID:	eB144

Format:	IgG
F/P Ratio:	2.3

Target Details

Reactivity:	Mouse
Purity/Specificity:	Fluorescent Mouse 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-Mouse Serum. Reactivity is observed against native Mouse IgG by both Western blot and ELISA.
Relevant Links:	• Fluorescent TrueBlot® Anti-Mouse Ig DyLight™ 800 IP Western Blot Protocol • 18-4517-32 SDS • DyLight™ Antibody Spectra

Application Details

Tested Applications:	IP, WB

Application Note:	Fluorescent Mouse TrueBlot® Antibody DyLight™ 800 has been tested in western blot and immunoprecipitation and may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescent Mouse 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 40 blots. Note that there are three key procedural considerations: 1. Protein A or G beads may be used with the mouse, goat and sheep TrueBlot secondaries, but not with the rabbit TrueBlot secondary. Use of protein A or G beads with the rabbit TrueBlot will result in contaminating bands. 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. Note: To achieve best results when detecting mouse IgG1 subtypes, we recommend performing a dot blot or titration to determine the optimal dilution factor for your desired application. 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

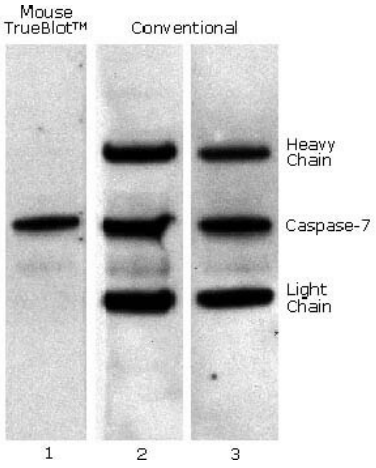
Physical State:	Lyophilized

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)

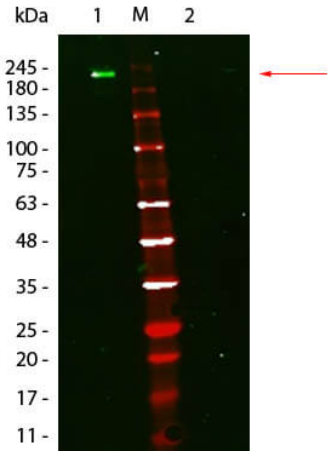
Shipping & Handling

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
Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1x10e7 Jurkat cells/ml with 5 ug mouse anti-human Caspase 7. Precipitate from 1x10e6 cells was subjected to electrophoresis, transferred to an PVDF membrane, and Western blotted with anti-Caspase 7 using Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (Lane 1) or conventional HRP-conjugated anti-mouse antibody (Lane 2) - note the detection of the heavy and light chains of the immunoprecipitating antibody in Lane 2 but not in Lane 1. When Lane 1 is re-immunoblotted using conventional HRP-conjugated anti-mouse polyclonal antibody (Lane 3), the heavy and light chains are now detected, confirming that although the immunoprecipitating heavy and light chains are present, Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP detects only native antibody and not denatured heavy and light chains.



Western Blot
Western Blot of Fluorescent TrueBlot®: Anti-Mouse Ig DyLight™ 800. Lane 1: Mouse IgG, Non-reduced. Lane 2: Mouse IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Fluorescent TrueBlot®: Anti-Mouse Ig 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 Mouse IgG, Non-reduced. Migrates at slightly higher molecular weight than predicted.

References

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