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Datasheet for 18-4417-32

Fluorescent TrueBlot®: Anti-Mouse Ig DyLight™ 680

Overview

Description:	Fluorescent TrueBlot®: Anti-Mouse Ig DyLight™ 680 - 18-4417-32
Item No.:	18-4417-32
Size:	100 µL
Applications:	ELISA, IF, IP, WB
Reactivity:	Mouse
Host Species:	Rat

Product Details

Background:	Mouse IgG TrueBlot® is a unique DyLight™ 680 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 DL680 Anti-mouse IgG blotting reagent with Fluorescent Mouse TrueBlot® Antibody DyLight™ 680 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 blotting applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.
Synonyms:	Anti-Mouse IgG DL680, TrueBlot, DL680 TrueBlot ULTRA, DyLight™ 680 TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Ms TrueBlot, IRDye 700, IRDye 680
Host Species:	Rat
Conjugate:	DyLight™ 680
Clonality:	Monoclonal
Clone ID:	eB144

Format:	IgG
F/P Ratio:	2.4

Target Details

Reactivity:	Mouse
Purity/Specificity:	Fluorescent Mouse TrueBlot® Antibody DyLight™ 680 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:	• 18-4417-32 SDS • Fluorescent TrueBlot® Anti-Mouse Ig DyLight™ 680 IP Western Blot Protocol • DyLight™ Antibody Spectra

Application Details

Tested Applications:	ELISA, IF, IP, WB
Application Note:	Fluorescent Mouse TrueBlot® Antibody DyLight™ 680 has been tested in ELISA, immunofluorescence, immunoprecipitation, and western blot and may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescent Mouse TrueBlot® Antibody DyLight™ 680 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. MB-070 Blocking Buffer for Fluorescent Western Blotting should be used as the blocking protein for the immunoblot. 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.
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

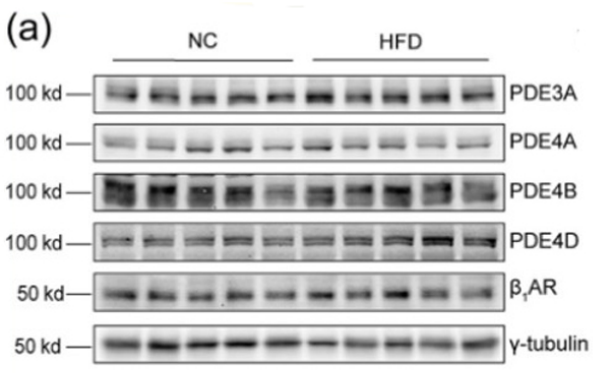
Formulation

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)

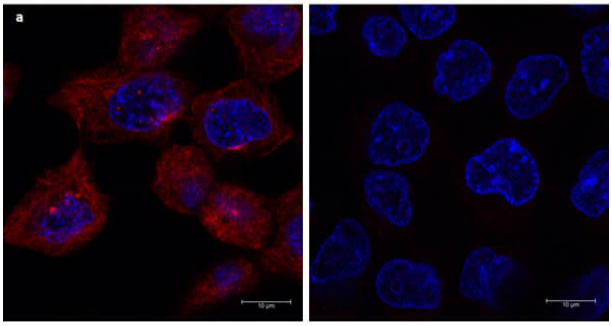
Shipping & Handling

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.

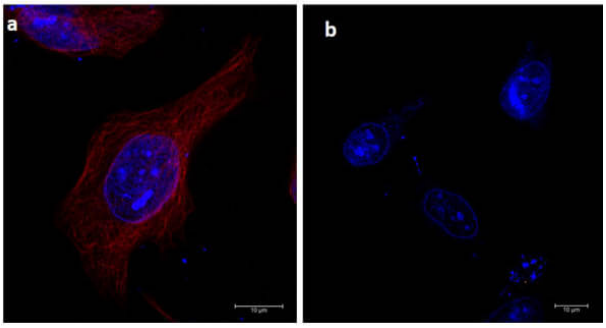
Images



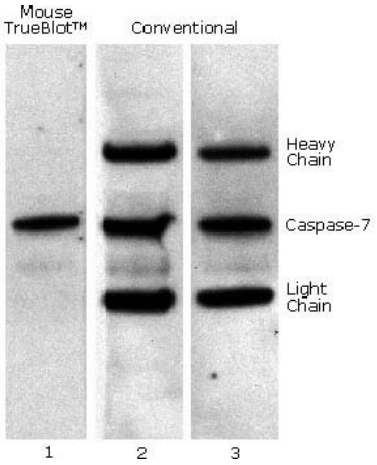
Western Blot
(a. WT mice were fed with a HFD or normal chow (NC) diet for 4.5 months. The heart tissues were harvested for western blot analysis to detect the expression of β1-adrenoceptor, PDE3A, 4A, 4B, 4D and γ-tubulin. Fluorescent TrueBlot®: Anti-Mouse IgG DyLight™ 680 (p/n 18-4417-32) and Fluorescent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



Immunofluorescence Microscopy
Immunofluorescence microscopy of a-tubulin in A431 cells using DyLight™ 680-conjugated Fluorescent TrueBlot® anti-mouse IgG for detection. A431 cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100 in 1X PBS) for 1 hr, then incubated with 15 µg/mL of anti-a-tubulin primary antibody (Cat. No. 200-301-880) at 4°C overnight. After 3 washes in 1X PBS for 5 min each, 5 µg/mL of Fluorescent TrueBlot® anti-mouse IgG DyLight™ 680 was added and allowed to incubate for 1 hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged a-tubulin (red)/DAPI (blue) image shown. (b) secondary antibody only.

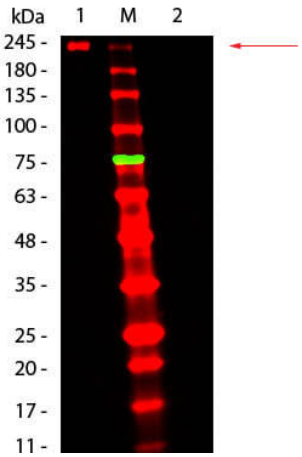


Immunofluorescence Microscopy
Immunofluorescence microscopy of α-tubulin in HeLa cells using DyLight™ 680-conjugated Fluorescent TrueBlot® anti-mouse IgG (p/n 18-4417-32) for detection. HeLa cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100 in 1X PBS) for 1hr, then incubated with 15µg/mL of anti-alpha-tubulin primary antibody (p/n 200-301-880) at 4°C overnight. Following 3 washes in 1X PBS for 5 min each, 5µg/mL of Fluorescent TrueBlot® anti-mouse IgG DyLight™ 680 was added and allowed to incubate for 1hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged a-tubulin (red)/DAPI (blue) image shown. (b) secondary antibody only.



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 680 Conjugated. 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 680 Conjugated 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. Other band(s): none.

References

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