Datasheet for ABIN6698838

Fluorescent TrueBlot®: Anti-Mouse Ig Fluorescein

3 Images

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Overview

Quantity:	100 µL
Target:	Ig
Reactivity:	Mouse
Host:	Rat
Conjugate:	FITC
Application:	Immunohistochemistry (IHC), Western Blotting (WB), FLISA, Flow Cytometry (FACS), Fluorescence Microscopy (FM)

Product Details

Brand:	TrueBlot®
Characteristics:	Synonyms: FITC, TrueBlot, FITC TrueBlot ULTRA, Fluorescein TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Ms TrueBlot Background: Mouse IgG TrueBlot® is a unique fluorescein conjugated Anti-mouse IgG immunoblotting (second step) reagent. 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 FITC Anti-mouse IgG blotting reagent with Fluorescent Mouse TrueBlot® Antibody Fluorescein 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

Product Details

	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.
Purification:	Fluorescent Mouse TrueBlot® Antibody Fluorescein Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography. Assay by Immunoelectrophoresis resulted in a single precipitin arc against anti-fluorescein and Anti-Mouse Serum. Reactivity is observed against native Mouse IgG by both Western blot and ELISA.
Labeling Ratio:	3.6

Target Details

Target:	Ig
Abstract:	Ig Products

Application Details

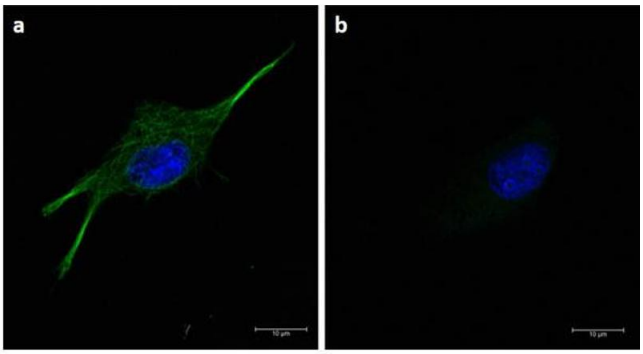
Application Notes:	Immunohistochemistry Dilution: User Optimized Application Note: Fluorescent Mouse TrueBlot® Antibody Fluorescein may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescein Conjugated Antibodies are designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms. Fluorescent Mouse TrueBlot® Antibody Fluorescein 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

Application Details

	specifically optimized by the end user. FLISA Dilution: User Optimized Flow Cytometry Dilution: User Optimized Western Blot Dilution: 1:1000 IF Microscopy Dilution: 1:200
Restrictions:	For Research Use only

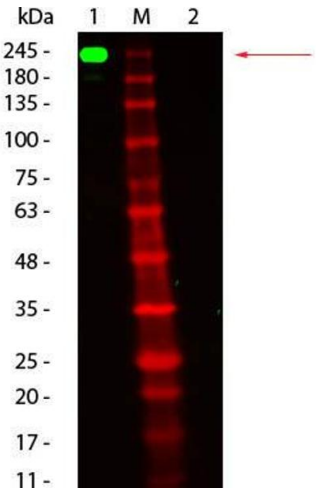
Handling

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
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



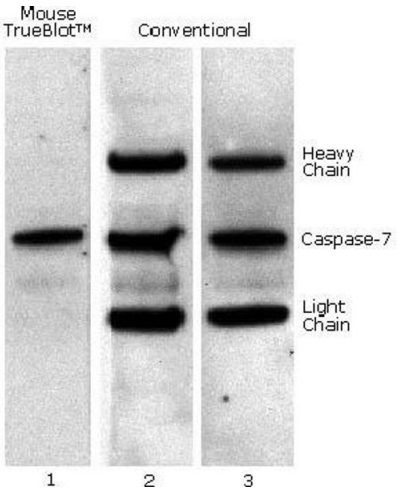
Immunofluorescence

Image 1. Immunofluorescence of α-tubulin using FITC-conjugated Fluorescent anti-mouse IgG Immunofluorescence microscopy of α-tubulin in U-87 MG cells using FITC-conjugated Fluorescent anti-mouse IgG for detection. U87-MG cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100) for 1hr, then incubated with 15µg/mL of anti-alpha-tubulin primary antibody at 4°C overnight. Following 3 washes in 1X PBS for 5min each, 5µg/mL of Fluorescent anti-mouse IgG Fluorescein was added and allowed to incubate for 1hr at room temperature. 5µg/mL of Fluorescent anti-mouse IgG FITC was added and allowed to incubate for 1hr at room temperature. Nucleus was counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged α-tubulin (green)/DAPI (blue) image shown (b) secondary only.



Western Blotting

Image 2. Fluorescein: Anti-Mouse Ig Fluorescein Conjugated Western Blot of Fluorescent: Anti-Mouse Ig Fluorescein. Lane 1: Mouse IgG, Non-reduced. Lane 2: Mouse IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Fluorescent: Anti-Mouse Ig Fluorescein at 1:1,000 for 60 min at RT. Block: ABIN925618 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.



Western Blotting

Image 3. Mouse 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 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 ULTRA: Anti-Mouse Ig HRP detects only native antibody and not denatured heavy and light chains.