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Datasheet for 18-0216-32

Fluorescent TrueBlot®: Anti-Rabbit IgG Fluorescein

Overview

Description:	Fluorescent TrueBlot®: Anti-Rabbit IgG Fluorescein - 18-0216-32
Item No.:	18-0216-32
Size:	100 µL
Applications:	IF, IP, WB
Reactivity:	Rabbit
Host Species:	Mouse

Product Details

Background:	Rabbit IgG TrueBlot® is a unique fluorescein 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 FITC Anti-rabbit IgG blotting reagent with Fluorescent Rabbit TrueBlot® Antibody Fluorescein and follow the prescribed protocol for sample preparation and immunoblotting. 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 FITC, TrueBlot, FITC TrueBlot ULTRA, Fluorescein TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Rb TrueBlot
Host Species:	Mouse
Conjugate:	Fluorescein (FITC)
Clonality:	Monoclonal
Clone ID:	eB182

Format:	IgG
F/P Ratio:	3.5

Target Details

Reactivity:	Rabbit
Purity/Specificity:	Fluorescent Rabbit 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-Rabbit Serum. Reactivity is observed against native Rabbit IgG by both Western blot and ELISA.
Relevant Links:	• Fluorescent TrueBlot® Anti-Rabbit IgG Fluorescein IP Western Blot Protocol

Application Details

Tested Applications:	IF, IP, WB
Application Note:	Rabbit IgG TrueBlot® Fluorescein Conjugated Antibody has been tested in immunofluorescence microscopy, fluorescent western blotting, and immunoprecipitation and are suitable for fluorescence based plate assays (FLISA, multiplex analysis, including multicolor imaging, utilizing various commercial platforms. Fluorescent Rabbit TrueBlot® Antibody Fluorescein may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescent Rabbit 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 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. MB-070 Blocking Buffer for Fluorescent Western Blotting should be used as the blocking protein for the immunoblot. 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

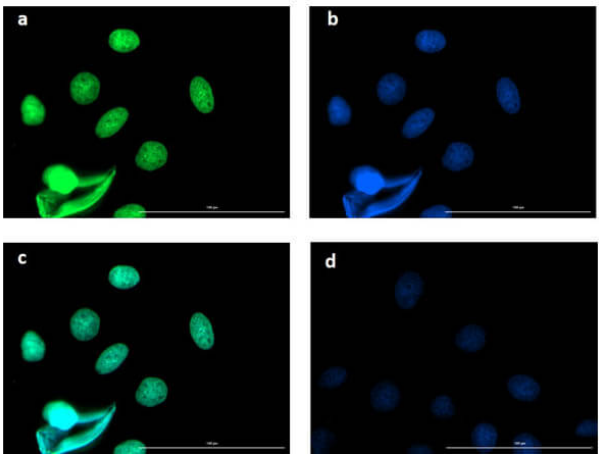
Formulation

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)

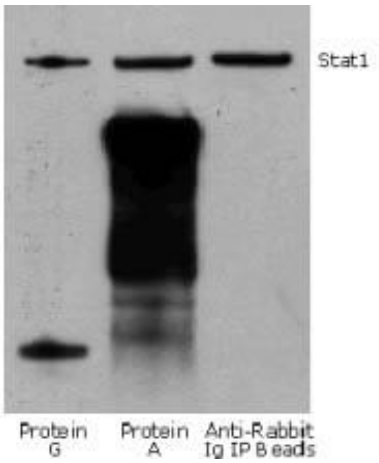
Shipping & Handling

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.

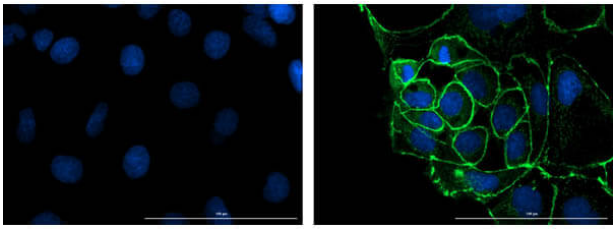
Images



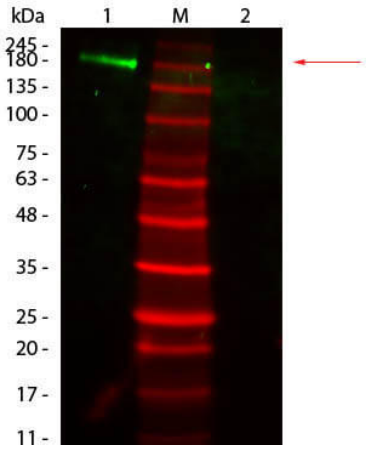
Immunofluorescence Microscopy
Immunofluorescence microscopy of BCL3 in Caco-2 cells using FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG for detection. Caco-2 cells were fixed with 4% PFA, blocked (5% mouse serum/0.3% Triton X-100 in 1X PBS) for 1 hr, then incubated with 15 µg/mL of anti-BCL3 primary antibody (Cat. No. 600-401-GU4) at 4°C overnight. Following 3 washes in 1X PBS for 5 min each, 5 µg/mL of FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG 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 nucleoplasm. Additional localization in some cell types includes vesicles and midbody. (a) BCL3 (b) DAPI (c) merged DAPI/BCL3 (d) secondary antibody only. Image taken at 40X magnification.



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



Immunofluorescence Microscopy
Immunofluorescence microscopy of ZO-1 in Caco-2 cells using FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG for detection. Caco-2 cells were fixed with 4% PFA, blocked (5% mouse serum/0.3% Triton X-100 in 1X PBS) for 1 hr, then incubated with 15 µg/mL of anti-ZO-1 primary antibody (Cat. No. 600-401-GU7) at 4°C overnight. Following 3 washes in 1X PBS for 5 min each, 5 µg/mL of FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG was added and allowed to incubate for 1 hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. Predicted cell localization is cell membrane and cell junctions. Image taken at 40X magnification. (right) Merged DAPI (blue)/ZO-1 (green), image shown (left) secondary antibody only.



Western Blot
Western Blot of Fluorescent TrueBlot®: Anti-Rabbit IgG Fluorescein. Lane 1: Rabbit IgG, Non-reduced. Lane 2: Rabbit IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Fluorescent TrueBlot®: Anti-Rabbit IgG Fluorescein 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. Other band(s): none.

References

- Zhang C et al. Anti-inflammatory effects of α-MSH through p-CREB expression in sarcoidosis like granuloma model. *Sci Rep.* (2020)

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