Fluorescent TrueBlot®: Anti-Mouse Ig Fluorescein - 18-0217-32

Code: 18-0217-32 **Size:** 100 µL

Product Description: Fluorescent TrueBlot®: Anti-Mouse Ig Fluorescein - 18-0217-32

Concentration: 1.0 mg/mL by UV absorbance at 280 nm

PhysicalState: Lyophilized

Label	Fluorescein (FITC)
Host	Rat
Emission Wavelength	528
Excitation Wavelength	495
Species Reactivity	Mouse
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Reconstitution Volume	100 µL
Reconstitution Buffer	Restore with deionized water (or equivalent)
Stabilizer	10 mg/ml Polyethylene Glycol (PEG-8000)
Preservative	0.01% (w/v) Sodium Azide
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.
Synonyms	FITC, TrueBlot, FITC TrueBlot ULTRA, Fluorescein TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Fluorescent TrueBlot, Ms TrueBlot
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 specifically optimized by the end user.
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 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.
Purity And Specificity	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.
Assay Dilutions	User Optimized
Western Blot	1:1000
FLISA	User Optimized

Immunohistochemistry	User Optimized
IF Microscopy	1:200
Flow Cytometry	User Optimized
Other Assays	User Optimized
Expiration	Expiration date is one (1) year from date of opening.

General Reference

Kong, D., L. Xu, Y. Yu, W. Zhu, D.W. Andrews, Y. Yoon, and T.H. Kuo. 2005. Regulation of Ca2+-induced permeability transition by BCL-2 is antagonized by Drp1 and hFis1. Molecular and Cellular Biochemistry. 272: 187-199. (Rabbit IgG TrueBlot, PubMed)

DiPerna, G., J. Stack, A.G. Bowie, A. Boyd, G. Kotwal, Z. Zhang, S. Arvikar, E. Latz, K.A. Fitzgerald, and W.L. Marshall. 2004. Poxvirus protein N1L targets the I-kappaB Kinase complex, inhibits signaling to NF-kappaB by the Tumor Necrosis Factor superfamily of receptors, and inhibits NF-kappaB and IRF3 signaling by Toll-like Receptors. J. Biol. Chem. 279: 36570-36578. (Rabbit IgG TrueBlot, PubMed)

Zhang, X., Y. Ozawa, H. Lee, Y. Wen, T. Tan, B. Wadzinski, and E. Seto. 2005. Histone deacetylase 3 (HDAC3) activity is regulated by interaction with protein serine/threonine phosphatase 4. Genes & Development. 19: 827-839. (Rabbit IgG TrueBlot, PubMed)

Lehtonen, S., E. Lehtonen, K. Kudlicka, H. Holthöfer, and M.G. Farquhar. 2004. Nephrin Forms a Complex with Adherens Junction Proteins and CASK in Podocytes and in Madin-Darby Canine Kidney Cells Expressing Nephrin. Am J Pathol. 165:923-936. (Rabbit IgG TrueBlot, PubMed)

Tyagi A, Agarwal C, Harrison G, Glode LM, Agarwal R. 2004. Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages. Carcinogenesis. 25: 1711-20. (Mouse IgG TrueBlot, PubMed)

Related Products

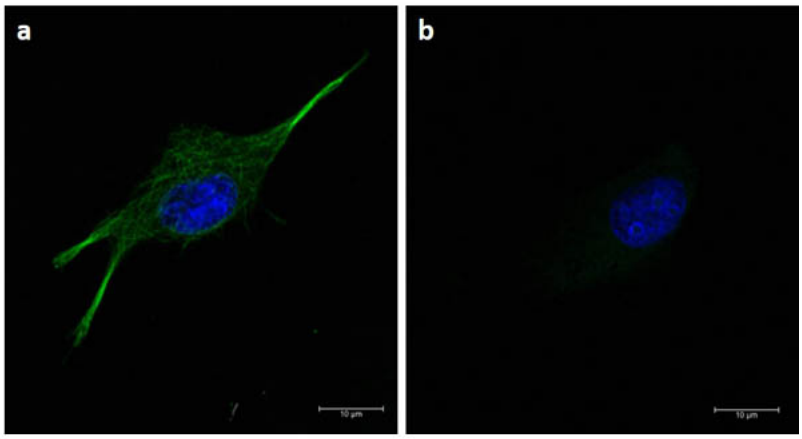
18-0216-32	Fluorescent TrueBlot®: Anti-Rabbit IgG Fluorescein18-0216-32
18-4417-32	Fluorescent TrueBlot®: Anti-Mouse IgG DyLight™ 68018-4417-32
18-8816-31	Rabbit TrueBlot®: Anti-Rabbit IgG HRP18-8816-31
18-8816-33	Rabbit TrueBlot®: Anti-Rabbit IgG HRP18-8816-33

Related Links

Fluorescent TrueBlot® Anti-Mouse Ig Fluorescein IP Western Blot Protocol

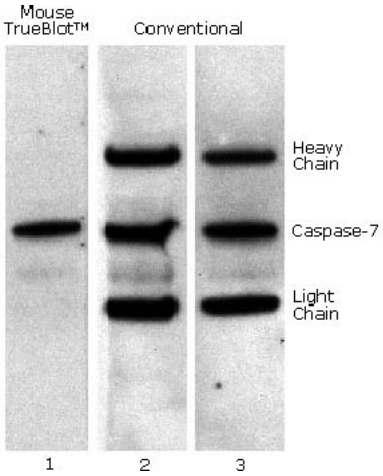
Images

1 Immunofluorescence microscopy of a-tubulin in U-87 MG cells using FITC-conjugated Fluorescent TrueBlot® anti-mouse IgG (p/n 18-0217-32) 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 (p/n 200-301-880) at 4°C overnight. Following 3 washes in 1X PBS for 5min each, 5µg/mL of Fluorescent TrueBlot® anti-mouse IgG Fluorescein was added and allowed to incubate for 1hr at room temperature. 5µg/mL of Fluorescent TrueBlot® 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 a-tubulin (green)/DAPI (blue) image shown (b) secondary only.



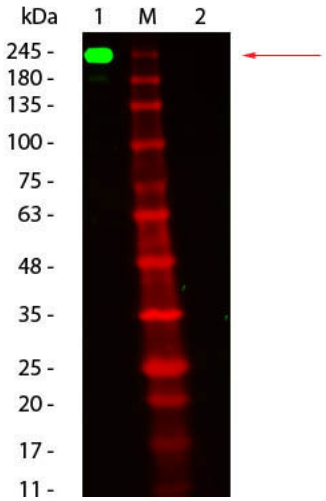
2

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.



3

Western Blot of Fluorescent TrueBlot®: 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 TrueBlot®: Anti-Mouse Ig Fluorescein 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.



Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.