



Mouse TrueBlot® Western Blot Kit - 88-8887-31

Code: 88-8887-31

Size: 1 Kit

Product Description: Mouse TrueBlot® Western Blot Kit - 88-8887-31

PhysicalState: Liquid (sterile filtered)

Label	HRP TrueBlot® ULTRA for IP/WB
Host	Rat
Species Reactivity	Mouse
Buffer	0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Stabilizer	0.1 mg/ml Bovine Serum Albumin (BSA) - IgG and Protease free, 50% (v/v) Glycerol
Storage Condition	Store Kit at 2-8 °C, except Mouse TrueBlot® ULTRA which should be stored at -20 °C. This product is guaranteed for 6 months upon receipt, when handled and stored as instructed.
Synonyms	HRP, TrueBlot, HRP TrueBlot ULTRA, Peroxidase TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, TrueBlot for chemiluminescent western blotting
Application Note	Mouse IgG TrueBlot® ULTRA is provided as 1000X solution. 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 20 blots. Mouse IgG TrueBlot® ULTRA. An HRP-conjugated second step reagent reacting with mouse IgGs for optimal signal detection in immunoprecipitation/immunoblotting experiments. 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. BLOTTO/Milk should be used as the blocking protein for the immunoblot. A sample of Anti-Alpha-Tubulin (MOUSE) Monoclonal Antibody Cat # (200-301-880S), has been provided to be used as a loading control at the user's discretion. Dilutions are to be optimized by the user. Mouse TrueBlot® Western Blot Kit Components: 1. Mouse IgG TrueBlot ULTRA: 50 L 18-8817-31, 2. TrueBlot Enhancer Solution: 25 mL; 3. TrueBlot Blocker: 10 g; 4. TrueBlot Assay Buffer: 30 mL, 20X; 5. TrueBlot Substrate A: 12.5 mL; 6. TrueBlot Substrate B: 12.5 mL; 7. Western Blot Incubation Tray. 8. Anti-Alpha-Tubulin (MOUSE) Monoclonal Antibody - 200-301-880S.
Background	The Mouse TrueBlot® Western Blot Kit contains the critical supporting reagents, buffers, and substrates for immunoprecipitation and Western blotting of samples using TrueBlot second step immunoblotting reagents in conjunction with your own primary IP antibody and primary (Mouse IgG) Western blotting antibody (see application note for kit components). Mouse IgG TrueBlot® ULTRA is the unique horseradish peroxidase conjugated Anti-Mouse IgG immunoblotting second step reagent which enables detection of immunoblotted target protein bands, without hindrance by interfering immunoglobulin heavy and light chains from your IP antibody. Use it in place of your conventional HRP Anti-Mouse IgG immunoblotting secondary antibody. It is easy to generate publication-quality IP/WB data with Mouse IgG TrueBlot® ULTRA. TrueBlot technology enables unhindered detection of protein bands of interest which would otherwise be obscured by the presence of reduced and denatured heavy and light chain immunoglobulin in the blot (as detected by the conventional immunoblotting HRP anti-mouse IgG reagent). Mouse IgG TrueBlot ULTRA 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 ULTRA 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/immunoblot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.
Purity And Specificity	Mouse TrueBlot® ULTRA Antibody Peroxidase 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.
Western Blot	1:1000
Expiration	Expiration date is six (6) months 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 Ca ²⁺ -induced permeability transition by BCL-2 is antagonized by Drp1 and hFis1. <i>Molecular and Cellular Biochemistry</i> . 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. <i>J. Biol. Chem.</i> 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. <i>Genes & Development</i> . 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. <i>Am J Pathol.</i> 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 CDK1-CDK-cyclin cascade, and caspase 3 and PARP cleavages. <i>Carcinogenesis</i> . 25: 1711-20. (Mouse IgG TrueBlot, PubMed)

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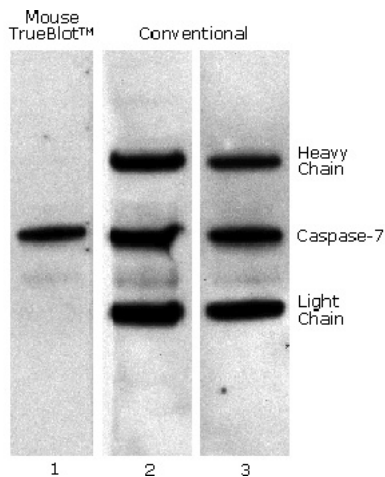
18-8817-30	Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP18-8817-30
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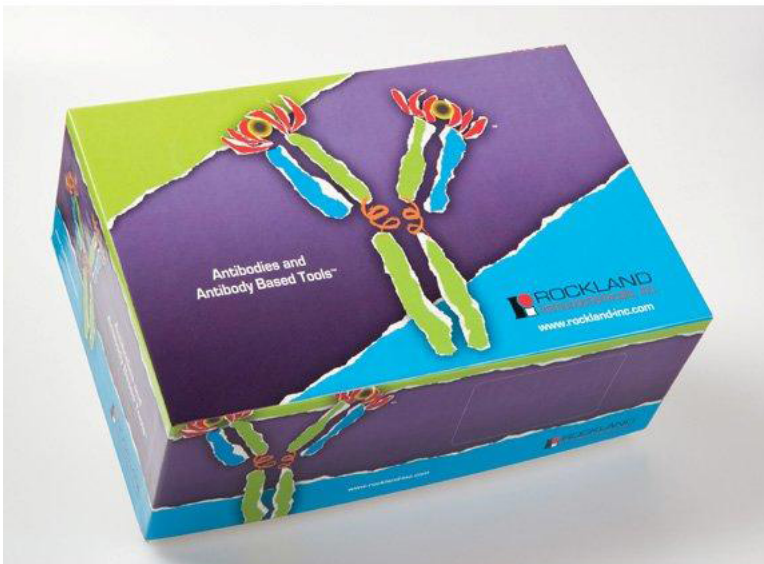
Mouse IgG TrueBlot / IP Western Blot Protocol

Images

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- Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1×10^7 Jurkat cells/ml with 5 μ g mouse anti-human Caspase 7. Precipitate from 1×10^6 cells was subjected to electrophoresis, transferred to a 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.



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- Rockland produces kits to meet the requirements of researchers for highly sensitive antibody based assays. Mouse TrueBlot® Western Blot Kit Components: 1. Mouse IgG TrueBlot ULTRA: 50 μ L 18-8817-31, 2. TrueBlot Enhancer Solution: 25 mL; 3. TrueBlot Blocker: 10 g; 4. TrueBlot Assay Buffer: 30 mL, 20X; 5. TrueBlot Substrate A: 12.5 mL; 6. TrueBlot Substrate B: 12.5 mL; 7. Western Blot Incubation Tray. 8. Anti-Alpha-Tubulin (MOUSE) Monoclonal Antibody - 200-301-880S.



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