Datasheet for ABIN1589983

Mouse TrueBlot® Western Blot Kit

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Overview

Quantity:	1 kit
Target:	IgG
Reactivity:	Mouse
Host:	Rat
Clonality:	Monoclonal
Conjugate:	HRP
Application:	Western Blotting (WB), Immunoprecipitation (IP)

Product Details

Brand:	TrueBlot®
Specificity:	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.
Characteristics:	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. 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 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 usual HRP anti-mouse IgG immunoblotting second step reagent. It is easy to generate publication-quality IP/WB data with Mouse IgG TrueBlot® ULTRA. 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/immunoblotting applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions. Conjugation Name: HRP TrueBlot® ULTRA
Purification:	Mouse TrueBlot® ULTRA Antibody Peroxidase Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography.
Sterility:	Sterile filtered
Components:	- Mouse IgG TrueBlot: 50 µL. An HRP-conjugated second step reagent reacting with mouse IgGs for optimal signal detection in immunoprecipitation/immunoblotting experiments (1000X) - TrueBlot® Enhancer Solution: 25 mL - TrueBlot® Blocker: 10 g - TrueBlot® Assay Buffer: 30 mL 20X - TrueBlot® Substrate A: 12.5 mL - TrueBlot® Substrate B: 12.5 mL - Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody - 25 µL. A primary monoclonal antibody has been provided to be used as a loading control at the user's discretion. Dilutions should be optimized by the user.
Material not included:	- Immobilized Protein A or Mouse Ig IP beads which is included in the kit (ABIN1589980) - Immunoprecipitation antibody - PVDF or nitrocellulose membrane (0.2 of 0.45 µm) - Immunoblotting mouse primary antibody - Chemiluminscent Substrate - X-ray film

Target Details

Target:	IgG
Abstract:	IgG Products
Target Type:	Antibody

Application Details

Application Notes:	Immunoprecipitation Dilution: Use with TrueBlot® Anti-Mouse Ig IP Beads (ABIN1589965) Western Blot Dilution: 1:1000
Comment:	Mouse IgG TrueBlot® ULTRA is provided as 1000X solution. In order to conserve reagent, we recommend incubating the blots from minigels in sealed bags (removing as much air as possible) with minimal volume (2-3 mL). If used conservatively at 2.5 mL/blot, cat. 18-8817-31 (50µL) 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 two key procedural considerations: 1. Immunoprecipitate should be completely reduced. 2. Milk should be used as the blocking protein for the immunoblot. The Mouse TrueBlot® Western Blot Kit components are sufficient for 20-25 miniblots. Components: 1. Mouse IgG TrueBlot ULTRA: 50 µl. 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
Sample Preparation:	Preparation of Cell Lysate 1. Harvest approximately 1x10^7 cells by using cell scraper and transfer to conical tube. If working with adherent cells you can skip this step and lyse directly on the plate (see Step 6) Note: The total number of cells per mL and the cell equivalent loaded per lane of gel should be optimized specifically for each protein and antibody. Alternatively, protein concentration can be determined using Bradford/Lowry or other protein assay. 2. Wash cells with 10 mL of cold PBS and centrifuge at 400 xg for 10 minutes at 4°C. 3. Discard the supernatant and repeat step 2. 4. After the second wash, remove the supernatant and resuspend the cell pellet in 1 mL of cold Lysis Buffer containing protease Inhibitors (such as a cocktail- see recipe below). Final concentration of cells should be about 1x10^7 cells/ml. Note: If using adherent cells, the cold Lysis Buffer can be added directly to the plate and put on a rocker at 4°C. Harvest by either scraping or collecting just the supernatant and proceed to Step 8. 5. Gently vortex/mix and transfer to 1.5 mL tube.

	6. Place on ice for 30 minutes, with occasional mixing. 7. Centrifuge at 10,000 xg for 15 minutes at 4°C. 8. Carefully collect the supernatant, without disturbing the pellet and transfer to a new clean tube and discard pellet. 9. The protein concentration can be determined by Bradford or other assay. Samples can be diluted to 1 µg/ µL. 10. The cell lysate can be frozen at this point for long-term storage at -80°C. Cell Lysate Preclearing 1. Resuspend the immobilized Protein A or Anti-Goat IgG bead slurry by gently vortexing. Remove 50 µL and wash in Lysis buffer or IP buffer, if different. Resupend in 50 µL IP buffer. 2. Add 500 µL of cell lysate (5x10^6 cells or 500µg protein) to the pre-equlibrated bead slurry to and incubate on a rocking platform or a rotator for 30-60 minutes at 4°C. 3. Centrifuge at 2,500 xg for 2-3 minutes at 4°C and transfer the supernatant to a new 1.5 mL tube. If any of the bead slurry has been transferred, centrifuge again and carefully transfer the supernatant to another fresh 1.5 mL tube.
Assay Procedure:	Procedure Step I: Immunoprecipitation 1. Add 1-10 µg of immunoprecipitation antibody to the tube containing the cold precleared cell lysate. Note: This concentration of monoclonal antibody is suggested as a starting point. Each investigator may desire to titrate the concentration of antibody and volume of cell lysate in preliminary experiments to determine the optimal conditions. e.g., 1-10 µg/10^7 cells/1 mL lysate. Typically, 2 µg is a sufficient amount of antibody to maximally immunoprecipitate most antigens in 1 mL of extract from 1x10^7 cells. Using as little IP antibody as possible minimizes potential contamination of SDS reduced sample with nonreduced immunoprecipitating antibody light chain. It is not recommended to use more than 10 µg (per mL) or a final of 5 µg per lane. 2. Incubate at 4°C for 1 hour on a rocking platform or a rotator. 3. Add at least 50 µL of pre-equilibrated bead slurry to capture the immune complexes. 4. Incubate for 1 hour or overnight at 4°C on a rocking platform or a rotator. Note: Step 1 and 3 can combined for a single incubation. 5. Centrifuge the tube at 2,500 xg for 30 seconds at 4°C. 6. Carefully remove supernatant completely and wash the beads 3-5 times with 500 µL of cold Lysis Buffer, centrifuging to pellet beads in between each wash. In order to minimize background, care should be given to remove the supernatant completely after each wash.

7. After the last wash, carefully aspirate supernatant and add 50 µL of 1X Laemmli sample buffer (or any equivalent SDS-PAGE sample loading Buffer) to bead pellet.
Note: Please take into consideration composition of the Loading buffer. Reducing agents can be added.
8. Vortex and heat to 90-100°C for 10 minutes.
9. Centrifuge at 10,000 xg for 5 minutes, collect supernatant carefully and load onto the gel.
10. Alternatively, the supernatant samples can be collected, transferred to clean tube and frozen at -80°C if the gel is to be run later.
11. Follow manufacturer's instructions for SDS-PAGE.

Procedure Step II: Immunoblotting (Western Blotting, WB)
1. Transfer proteins from the gel onto PVDF or nitrocellulose membrane following instructions provided by the transfer system manufacturer for best protein transfer results.
2. Optional: To determine whether the proteins have been transferred to membrane, stain with a 0.1% Ponceau S solution. Protein bands can be visualized after staining for 5 minutes. To remove the Ponceau S stain, rinse with distilled water or TBS-T until most of the dye is removed before moving on to blocking step. Residual dye will not affect subsequent steps.
3. Remove membrane and soak in transfer buffer.
4. Under chemical hood, place the membrane in TrueBlot® Enhancer Solution and soak for 2minutes, then wash with TBS-T.
5. Place the membrane into the 5% TrueBlot Blocker in TrueBlot® Assay Buffer (enough to cover the membrane) and incubate for 2 hours at room temperature or overnight at 4°C on a rocking platform. [Preparation of 5% TrueBlot Blocker in TrueBlot Assay Buffer: Dilute 20X TrueBlot Assay Buffer with dH20 to 1X. Using TrueBlot® Blocker Powder, make a 5% (w/v) solution.]
Note: it is recommended to use Milk as the blocking reagent as BSA does not effectively block the reduced Ig chain recognition.
6. Remove the blocking buffer and rinse blot with TBS-T.
7. Prepare the primary goat immunoblotting antibody in Blocking Buffer as recommended by the supplier. If the recommended concentration is not known use a standard concentration of 1-2 µg/ml. If using hybrid- oma tissue culture supernatant or serum for immunoblotting, preliminary experiments should be performed to evaluate whether dilution of the supernatant or serum is needed for best results.
8. Incubate the blot with primary antibody for at least 2 hours at room temperature of overnight at 4°C on rocking platform.
Note: Shorter times should be determined empirically for optimal results

Application Details

	9. After the overnight incubation of the membrane with the primary antibody, wash the blot at least 3-5 times in TBS-T, each wash for a minimum of 5-10 minutes each. Total should be more than 1 hour. 10. Prepare the secondary antibody Goat IgG TrueBlot® at a 1:1,000 dilution in the Blocking Buffer. Note: Please avoid the presence of sodium azide in this step as it is deleterious to the HRP enzyme. 11. Incubate the blot with the TrueBlot® secondary antibody for one hour at room temperature on a rocking platform. 12. Wash the blot at least 3-5 times in TBS-T, each wash for at least 5 minutes each. Total should be more than 1 hour. 13. Prepare Substrate: Mix equal volumes of Substrate A and B 14. Incubate the blot in chemiluminescent-HRP substrate working solution (combined A and B) for 1- 0.5 minutes. 15. Expose the blot to X-ray film for an appropriate time period. For best results, expose for ten seconds, one minute, five minutes and 20 minutes.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	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:	4 °C
Storage Comment:	Store 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.
Expiry Date:	6 months

Publications

Product cited in:	Kong, Xu, Yu, Zhu, Andrews, Yoon, Kuo: "Regulation of Ca2+-induced permeability transition by Bcl-2 is antagonized by Drpl and hFis1." in: **Molecular and cellular biochemistry**, Vol. 272, Issue 1-2, pp. 187-99, (2005) (PubMed). Zhang, Ozawa, Lee, Wen, Tan, Wadzinski, Seto: "Histone deacetylase 3 (HDAC3) activity is

regulated by interaction with protein serine/threonine phosphatase 4." in: **Genes & development**, Vol. 19, Issue 7, pp. 827-39, (2005) (PubMed).

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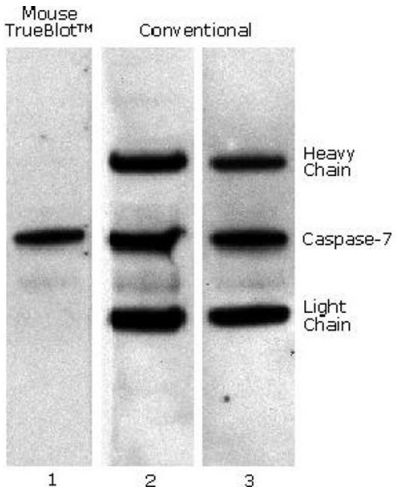
Lehtonen, Lehtonen, Kudlicka, Holthöfer, Farquhar: "Nephrin forms a complex with adherens junction proteins and CASK in podocytes and in Madin-Darby canine kidney cells expressing nephrin." in: **The American journal of pathology**, Vol. 165, Issue 3, pp. 923-36, (2004) (PubMed).

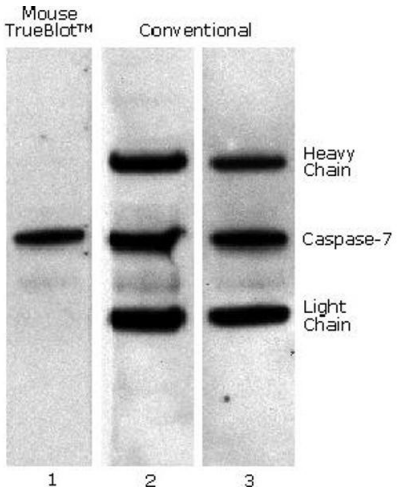
Tyagi, Agarwal, Harrison, Glode, Agarwal: "Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages." in: **Carcinogenesis**, Vol. 25, Issue 9, pp. 1711-20, (2004) (PubMed).

Images



Western Blotting

Image 1. Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1x10e7 Jurkat cells/mL with 5 µg 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 Blotting

Image 2. 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.