Datasheet for ABIN1589977

Mouse TrueBlot® ULTRA Anti-Mouse Ig HRP

3 Images | 73 Publications

Go to Product page

Overview

Quantity:	200 µL
Reactivity:	Mouse
Host:	Rat
Clonality:	Monoclonal
Conjugate:	HRP
Application:	Immunoprecipitation (IP), Western Blotting (WB)

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:	Mouse IgG TrueBlot® ULTRA is a unique anti-mouse IgG immunoblotting (second step) reagent. Mouse IgG TrueBlot® ULTRA 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/Western B blot data with Mouse IgG TrueBlot® ULTRA, simply substitute the conventional anti-Mouse IgG blotting reagent with Mouse IgG TrueBlot® ULTRA and follow the prescribed protocol for sample preparation and immunoblotting. Mouse IgG TrueBlot® ULTRA is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. Mouse IgG TrueBlot® ULTRA preferentially detects the non-reduced form of mouse IgG (IgG1, IgG2a, IgG2b, IgG3) over the reduced, SDS-denatured form of

Product Details

	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. Mouse TrueBlot® ULTRA has been reported for use in immunoblotting (WB). Conjugation Name: HRP TrueBlot® ULTRA
Purification:	MouseTrueBlot® ULTRA Antibody Peroxidase Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography.
Sterility:	Sterile filtered
Components:	Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP

Application Details

Application Notes:	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. 18-8817-30 (50uL) will yield enough reagent for 8 blots.
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 -84 °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 500 µg 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 1. 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 3. 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
1. 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 90-104 °C for
1. 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 -84 °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 2 minutes, then wash with TBS-T.
5. Place the membrane into the 1 % 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 1 % TrueBlot Blocker in TrueBlot Assay Buffer: Dilute 20X TrueBlot Assay Buffer with dH20 to 1X. Using TrueBlot® Blocker Powder, make a 1 % (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.

Application Details

	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 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 2 minutes.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 10 mg/mL Bovine Serum Albumin (BSA) - IgG and Protease free, 50 % (v/v) Glycerol
Handling Advice:	Use of Sodium Azide will inhibit enzyme activity of horseradish peroxidase.
Storage:	-20 °C
Storage Comment:	Store 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:	Guarnieri, Towers, Drasin, Oliphant, Andrysik, Hotz, Vartuli, Linklater, Pandey, Khanal, Espinosa,

Publications

Ford: "The miR-106b-25 cluster mediates breast tumor initiation through activation of NOTCH1 via direct repression of NEDD4L." in: **Oncogene**, Vol. 37, Issue 28, pp. 3879-3893, (2019) (PubMed).

Kistler, Trcek, Hurd, Chen, Liang, Sall, Kato, Lehmann: "Phase transitioned nuclear Oskar promotes cell division of Drosophila primordial germ cells." in: **eLife**, Vol. 7, (2019) (PubMed).

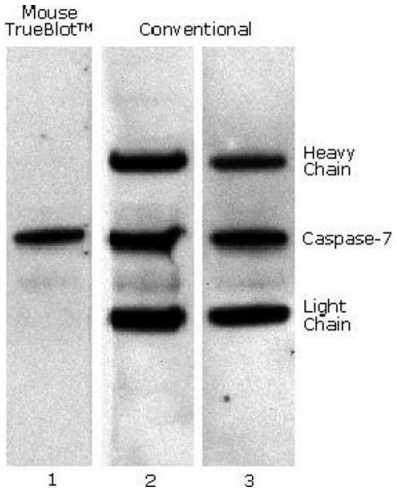
Menzies, Volkmar, van den Boomen, Timms, Dickson, Nathan, Lehner: "The sterol-responsive RNF145 E3 ubiquitin ligase mediates the degradation of HMG-CoA reductase together with gp78 and Hrd1." in: **eLife**, Vol. 7, (2019) (PubMed).

Mitchell, Barreyro, Todorova, Taylor, Antony-Debré, Narayanagari, Carvajal, Leite, Piperdi, Pendurti, Mantzaris, Paietta, Verma, Gritsman, Steidl: "IL1RAP potentiates multiple oncogenic signaling pathways in AML." in: **The Journal of experimental medicine**, Vol. 215, Issue 6, pp. 1709-1727, (2019) (PubMed).

Mehto, Jena, Nath, Chauhan, Kolapalli, Das, Sahoo, Jain, Taylor, Chauhan: "The Crohn's Disease Risk Factor IRGM Limits NLRP3 Inflammasome Activation by Impeding Its Assembly and by Mediating Its Selective Autophagy." in: **Molecular cell**, Vol. 73, Issue 3, pp. 429-445.e7, (2019) (PubMed).

There are more publications referencing this product on: Product page

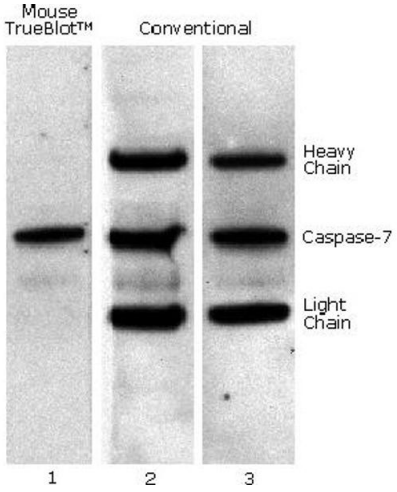
Images



Western Blotting

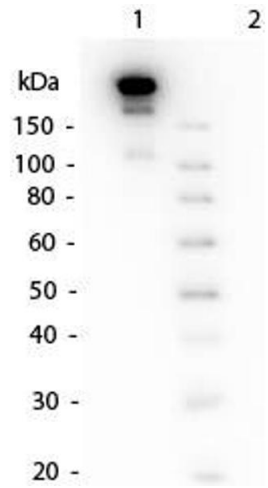
Image 1. Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1x10e7 Jurkat cells/mL with 5 µg mouse anti-human Caspase 7. Precipitate from 1x10e6 cells was subjected to electrophoresis, transferred to an PVDF membrane, and Western blotted with anti-Caspase 7 using Mouse 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.



Western Blotting

Image 3. Mouse TrueBlot - Western Blot 2 Western Blot of Mouse ULTRA: Anti-Mouse Ig HRP. Lane 1: Mouse IgG, non-denatured. Lane 2: 5 µL Opal Pre-stained Ladder . Lane 3: Mouse IgG, denatured. Load: 50 ng. Primary antibody: none. Secondary antibody: Mouse ULTRA: Anti-Mouse Ig HRP at 1:1,000 for 60 min at RT. Block: ABIN925618 for 30 min at RT. Predicted/Observed size: 160 kDa non-denatured for Mouse IgG.