Datasheet for ABIN1589974

Rabbit TrueBlot® Anti-Rabbit IgG HRP

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Overview

Quantity:	200 µL
Reactivity:	Rabbit
Host:	Mouse
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-Rabbit Serum. Reactivity is observed against native Rabbit IgG by both Western blot and ELISA.
Characteristics:	Rabbit IgG TrueBlot® is a unique horseradish peroxidase conjugated anti-rabbit IgG immunoblotting (second step) reagent. 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/Western Blot data with Rabbit IgG TrueBlot®, simply substitute the conventional HRP anti-rabbit IgG blotting reagent with Rabbit IgG TrueBlot® 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

Product Details

	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 blotting applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions. Rabbit IgG TrueBlot may also be used for detection in immunoblotting assays that do not employ immunoprecipitation. Conjugation Name: HRP TrueBlot® ULTRA
Purification:	Rabbit TrueBlot® Antibody Peroxidase Conjugate was prepared from tissue culture supernatant by Protein G affinity chromatography.
Sterility:	Sterile filtered
Components:	Rabbit TrueBlot®: Anti-Rabbit IgG HRP

Application Details

Application Notes:	Western Blot Dilution: 1:1000
Comment:	Rabbit IgG TrueBlot® 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. 18-8816-31 (50µL) will yield enough reagent for 20 blots. Note that there are three key procedural considerations: 1. Protein A or G should not be used for the immunoprecipitation. For immunoprecipitation, anti-mouse IgG beads, anti-rat IgG beads, or anti-rabbit IgG beads should be used for mouse, rat, or rabbit immunoprecipitating antibodies, respectively. 2. Immunoprecipitate should be completely reduced. 3. Milk should be used as the blocking protein for the immunoblot.
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.

Application Details

	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 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:	Buffer: 0.02 M Potassium 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 Proclin
Preservative:	ProClin
Precaution of Use:	This product contains ProClin: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Handling

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:

Kumar, Jain, Farzam, Jia, Gu, Choi, Mudd, Claude-Taupin, Wester, Lidke, Rusten, Deretic: " Mechanism of Stx17 recruitment to autophagosomes via IRGM and mammalian Atg8 proteins." in: **The Journal of cell biology**, Vol. 217, Issue 3, pp. 997-1013, (2019) (PubMed).

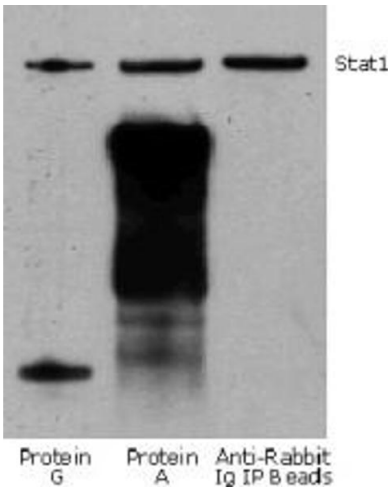
Cheng, Spengler, Keiser, Monteys, Rieders, Ramachandran, Davidson: "The long non-coding RNA NEAT1 is elevated in polyglutamine repeat expansion diseases and protects from disease gene-dependent toxicities." in: **Human molecular genetics**, Vol. 27, Issue 24, pp. 4303-4314, (2019) (PubMed).

Pazos, Peters, Casanova, Palacios, VanNieuwenhze, Breukink, Vicente, Vollmer: "Z-ring membrane anchors associate with cell wall synthases to initiate bacterial cell division." in: **Nature communications**, Vol. 9, Issue 1, pp. 5090, (2019) (PubMed).

Prevost, Pinotsis, Dumoux, Hayward, Waksman: "The Legionella effector WipB is a translocated Ser/Thr phosphatase that targets the host lysosomal nutrient sensing machinery." in: **Scientific reports**, Vol. 7, Issue 1, pp. 9450, (2019) (PubMed).

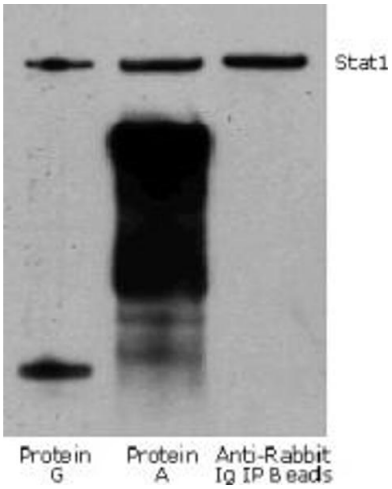
Short, Kondo, Smalley-Freed, Takeda, Dohn, Powell, Carnahan, Washington, Tripathi, Payne, Jenkins, Copeland, Coffey, Reynolds: "p120-Catenin is an obligate haploinsufficient tumor suppressor in intestinal neoplasia." in: **The Journal of clinical investigation**, Vol. 127, Issue 12, pp. 4462-4476, (2019) (PubMed).

There are more publications referencing this product on: Product page



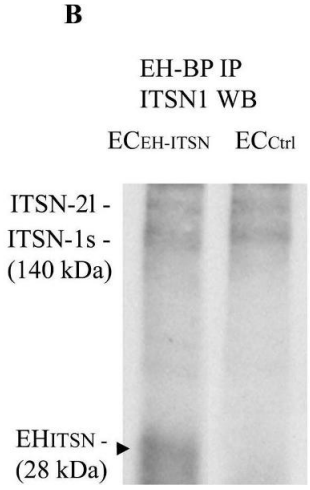
Western Blotting

Image 1. 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



Western Blotting

Image 2. Rabbit 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: Anti-Rabbit IgG HRP



Western Blotting

Image 3. Intersectin-1s (ITSN) interacts via the EH domains with the EHBP1. ECs lysates (250 µg total protein) were subjected to immunoprecipitation with anti-EHBP1 Ab (1 µ g), followed by WB with EHBP1 (A) and ITSN1 (B) Abs. EHBP1 Ab brings down the EHBP1 protein as well as ITSN. The 55 kDa immunoreactivity in panel A is cross-reactivity with the IgG heavy chain. The EHBP1 Ab immunoprecipitates the Myc-EHITSN from the stable transfected ECEH-ITSN lysates (B, arrowhead). (C). ECs lysates (250 µg total protein) were subjected to immunoprecipitation with anti-ITSN1 Ab (1 µg), followed by WB with ITSN1 Ab. ITSN1 Ab brings down ITSN protein in both ECEH-ITSN and ECCtrl lysates. The upper ITSN immunoreactivity (190 kDa), belongs to the ITSN-2 long

isoform (ITSN-2l). For immunoprecipitation studies (B,C), the rabbit IgG TrueBlot Ab HRP-conjugated which enables detection of immunoblotted target protein bands, without interfering with the immunoprecipitating IgG heavy and light chains has been used. (D) Densitometric analysis of immunoprecipitated ITSN in both ECEH-ITSN and ECCtrl lysates. Data are expressed as ratio of ITSN immunoprecipitated by EHBP1 Ab / ITSN immunoprecipitated by ITSN Ab (D). $p < 0.05$. (E,F). Double anti-ITSN Ab anti-rabbit IgG Alexa Fluor 594-conjugated (E) / anti-EHBP1 Ab - anti mouse IgG Alexa Fluor 488-conjugated (F). The merged image reveals significant co-localization ITSN/EHBP1, both in the cytosol and at the plasma membrane (G). (H) The magnification of the boxed area in G, highlights the significant co-localization ITSN/EHBP1 at the plasma membrane level (arrows) and cytosol (arrowheads). Bars: 10 µm (E-G), 5 µm (H), n = 5. - figure provided by CiteAb. Source: PMID30333761

Please check the product details page for more images. Overall 4 images are available for ABIN1589974.