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Datasheet for 18-8814-33

Goat TrueBlot® ULTRA: Anti-Goat IgG HRP

Overview

Description:	Goat TrueBlot® ULTRA: Anti-Goat IgG HRP - 18-8814-33
Item No.:	18-8814-33
Size:	200 µL
Applications:	IP, WB
Reactivity:	Goat
Host Species:	Mouse

Product Details

Background:	Goat IgG TrueBlot® is a unique horseradish peroxidase conjugated Anti-Goat IgG monoclonal secondary antibody. Goat 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 Goat IgG TrueBlot®, simply substitute the conventional HRP Anti-Goat IgG blotting reagent with Goat IgG TrueBlot® and follow the prescribed protocol for sample preparation and immunoblotting. Goat IgG TrueBlot® is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. TrueBlot preferentially detects the non-reduced form of goat IgG over the reduced, SDS-denatured form of IgG. When the immunoprecipitate is fully reduced immediately prior to SDS-gel electrophoresis, reactivity of Goat 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/immunoblot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.
Synonyms:	Anti-Goat IgG HRP, TrueBlot, HRP TrueBlot ULTRA, Peroxidase TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting
Host Species:	Mouse
Conjugate:	Peroxidase (HRP) ULTRA
Clonality:	Monoclonal
Clone ID:	eB270
Format:	IgG

Target Details

Reactivity:	Goat
Purity/Specificity:	Goat TrueBlot® 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-Goat Serum. Reactivity is observed against native Goat IgG by both Western blot and ELISA.
Relevant Links:	• TrueBlot HRP Product Protocols • TrueBlot IP Set Protocol • SDS

Application Details

Tested Applications:	IP, WB
Application Note:	Goat IgG HRP TrueBlot has been tested in western blot and immunoprecipitation and may also be used for detection in immunoblotting assays that do not employ immunoprecipitation. Goat IgG TrueBlot® 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 80 blots. 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. 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
WB:	1:1000

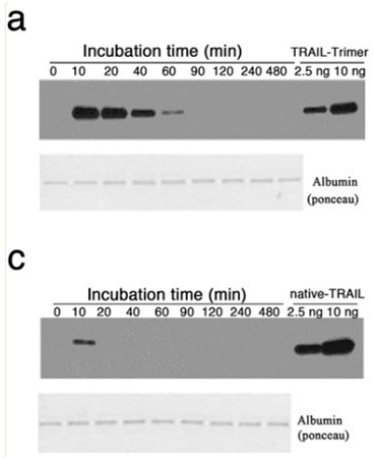
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1000X (see application note, titrated reagent enough for 80 blots)
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

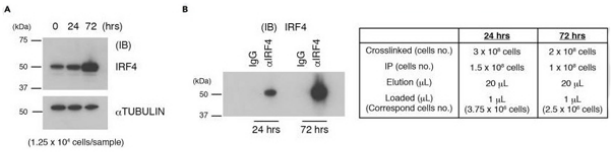
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store at -20°C. This product is guaranteed for 6 months upon receipt, when handled and stored as instructed.
Expiration:	Expiration date is six (6) months from date of receipt.

Images

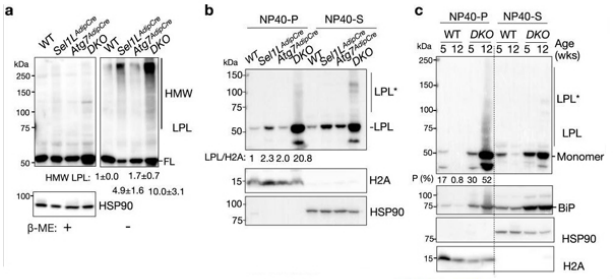


Western Blot
Pharmacokinetic profile of TRAIL-Trimer and native TRAIL determined by western blot. Mice were injected intravenously with equimolar amounts of TRAIL-Trimer (40 mg/kg) or native TRAIL (15 mg/kg) (n = 3 for each protein). (a,c) Relative serum concentrations of both TRAIL-Trimer (a) and native TRAIL (c) were evaluated at periodic intervals by western blotting. TRAIL proteins were immunoblotted with an antibody against human TRAIL, and visualized by chemiluminescence with HRP conjugated TrueBlot anti-goat IgG (1:2000 dilution) (p/n 18-8814-33). The images were acquired using the ChemiDoc Touch Imaging System (Bio-Rad) and the quantification of the amount of TRAIL proteins present in each sample was done using Image Lab software (Bio-Rad). Fig 5. PMID: 28827692



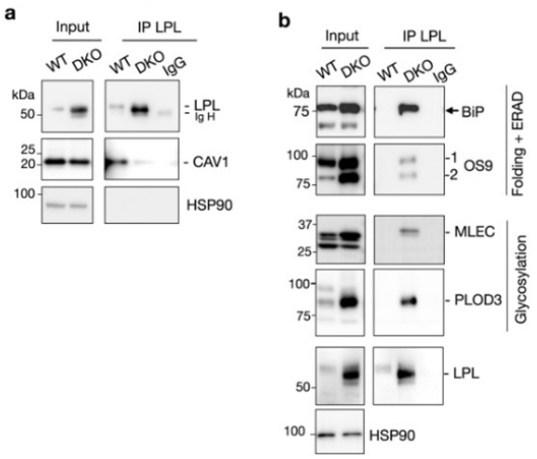
	24 hrs	72 hrs
Crosslinked (cells no.)	3 x 10⁶ cells	2 x 10⁶ cells
IP (cells no.)	1.5 x 10⁶ cells	1 x 10⁶ cells
Elution (µL)	20 µL	20 µL
Loaded (µL) (Correspond cells no.)	1 µL (3.75 x 10⁴ cells)	1 µL (2.5 x 10⁴ cells)

Immunoprecipitation
An example of IP evaluation.
(A) Immunoblot analysis of IRF4 protein expression in BCR activated B1-8hi splenic B cells at indicated time. αTUBULIN; internal control. 1.25 × 104 cells were loaded per sample.
(B) Evaluation of IRF4 IP 24 and 72 h post BCR activation. Left: Immunoblot analysis using αIRF4 antibodies for IP. IgG; control IgG. Goat TrueBlot was used for secondary antibody staining. To detect IP samples in IB, the TrueBlot series (Rockland) reduced detection of immunoglobulin heavy and light chains. (Right) Table indicates the details of IP procedure and sample amounts loaded for immunoblot analysis.



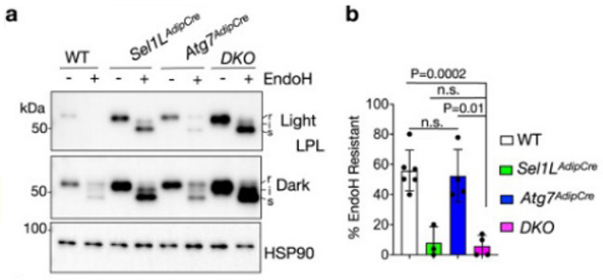
Western Blot

a) Immunoblot analysis of LPL under reducing (left) and non-reducing (right) conditions in gonadal WAT lysates of 12-week-old mice (n = 4 mice per WT and DKO, and 2 mice per Sel1LAdipCre and Atg7AdipCre). Stats shown comparing DKO vs. Sel1LAdipCre (P = 0.01) by one-way ANOVA followed by Tukey's test. b) Immunoblot analysis of LPL in gonadal WAT lysates of 12-week-old mice fractionated in 1% NP40 (S, soluble; P, pellet). HSP90 and H2A, loading controls for S and P fractions, respectively (n = 3 mice per genotypes). c) Immunoblot analysis of LPL in gonadal WAT lysates of 5- and 12-week-old WT and DKO mice fractionated in 1% NP40 as in b (n = 3 mice per group). LPL*: insoluble LPL that is resistant to heat and β-Mercaptoethanol (β-ME).
Fig 6. PMID: 37253728



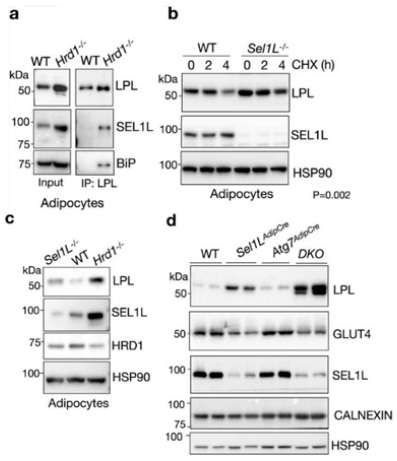
Western Blot

a, b) Immunoblot analysis following immunoprecipitation of endogenous LPL in gonadal WAT (n = 3 mice each).
Fig 5. PMID: 37253728



Western Blot

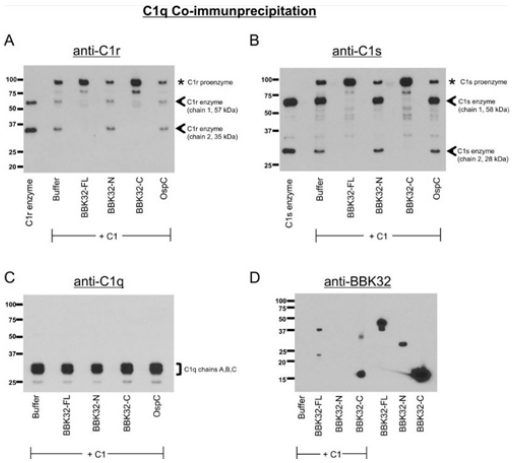
a, b) EndoH analysis of LPL in gonadal WAT using Western blot with quantitation of percentage of EndoH resistant LPL in (b) (n = 6 for WT, 3 for Sel1LAdipCre, 4 for Atg7AdipCre and DKO). s, i and r, EndoH sensitive, intermediate and resistant, respectively.
Fig 4. PMID: 37253728



Western Blot

a) Immunoblot analysis following immunoprecipitation of endogenous LPL in differentiated adipocytes (3 independent repeats). b) Immunoblot analysis of LPL in differentiated adipocytes pre-treated with Brefeldin A for 30 min followed by cycloheximide (CHX) treatment for the indicated times (2 independent repeats). c) Immunoblot analysis of LPL in differentiated adipocytes (3 independent repeats). d) Immunoblot analysis of LPL and other proteins in gonadal WAT.

Fig 3. PMID: 37253728



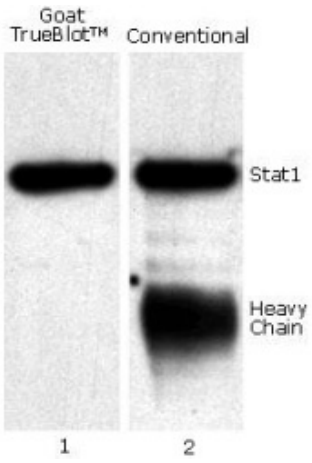
Immunoprecipitation

BBK32 inhibits the autocatalysis of C1r within the C1 complex.

C1 (40 nM) was incubated at 37°C for 2 hours in the presence or absence of 5 µM BBK32 or OspC proteins. Reactions were co-immunoprecipitated using a C1q monoclonal antibody previously adsorbed to protein G beads, and bound fractions were subjected to Western immunoblot analysis. (A) Reactions were probed with C1r polyclonal antibody. Previously purified activated C1r enzyme is loaded as a reference. Activated C1r is detected in buffer only reactions as judged by the presence of C1r enzyme chain 1 (57 kDa) and chain 2 (35 kDa), denoted by arrowheads. Reactions incubated with BBK32-FL or BBK32-C lack processed C1r and contained only the 92 kDa C1r proenzyme form (denoted by an asterisk). BBK32-N and a negative control protein (OspC) reactions contain processed C1r at levels indistinguishable from the buffer only reaction. (B) Reactions were probed with a C1s polyclonal antibody and previously purified activated C1s is loaded for reference. As was observed for C1r, reactions incubated with BBK32-FL or BBK32-C lack activated C1s and contain only C1s proenzyme (asterisk). Buffer only, BBK32-N, and OspC reactions contain equal amounts of activated C1s as judged by the presence of C1s enzyme chain 1 (58 kDa) and chain 2 (28 kDa) (denoted with arrowheads). (C) Detection with C1q polyclonal antibody indicates equivalent amounts of C1q are pulled down in all reactions. (D) Detection with BBK32 polyclonal antibody demonstrates that BBK32-FL or BBK32-C but not BBK32-N are pulled down with the C1 complex. The last three rightmost lanes contain 100 ng of each form of BBK32 protein as a reference.

The components of the C1 complex or BBK32 were detected with polyclonal goat anti-human C1q (diluted 1:5000), goat anti-human C1r (diluted 1:3000), sheep anti-human C1s (diluted 1:3000), or rabbit polyclonal antibody to BBK32 (diluted 1:1500) followed by incubation with either TrueBlot® ULTRA: HRP goat or sheep immunoglobulin (p/n 18-8814-31 or 18-8815-31) diluted 1:2000 (for the C1 proteins) or goat anti-rabbit IgG HRP diluted 1:5000 (for BBK32).

Fig 7. PMID: 26808924



Western Blot
Goat TrueBlot® IP / Western Blot: Jurkat cell lysate (0.5 ml of 1x10e7 cells/ml) was incubated with goat anti-human Stat1 and immunoprecipitated using Protein G. Precipitate from 5x10e5 cells was subjected to electrophoresis, transferred to a PVDF membrane, and Western blotted with anti-Stat1 using Goat TrueBlot® ULTRA: Anti-Goat IgG HRP (lane 1) and conventional HRP-conjugated anti-goat polyclonal antibody (lane 2).

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