

Datasheet for 18-8815-31**Sheep TrueBlot® ULTRA: Anti-Sheep IgG HRP****Overview**

Description:	Sheep TrueBlot® ULTRA: Anti-Sheep IgG HRP - 18-8815-31
Item No.:	18-8815-31
Size:	50 µL
Applications:	IP, WB
Reactivity:	Sheep
Host Species:	Mouse

Product Details

Background:	Sheep IgG TrueBlot® is a unique horseradish peroxidase conjugated Anti-Sheep IgG monoclonal secondary antibody. Sheep 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 Sheep IgG TrueBlot®, simply substitute the conventional HRP Anti-Sheep IgG blotting reagent with Sheep IgG TrueBlot® and follow the prescribed protocol for sample preparation and immunoblotting. Sheep IgG TrueBlot® is ideal for use in protocols involving immunoblotting of immunoprecipitated proteins. TrueBlot preferentially detects the non-reduced form of Sheep IgG over the reduced, SDS-denatured form of IgG. When the immunoprecipitate is fully reduced immediately prior to SDS-gel electrophoresis, reactivity of Sheep 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 blot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.
Synonyms:	Anti-Sheep 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:	Sheep
Purity/Specificity:	Sheep 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-Sheep Serum. Reactivity is observed against native Sheep IgG by both Western blot and ELISA.
Relevant Links:	TrueBlot HRP Secondary Antibodies Protocol

Application Details

Tested Applications:	IP, WB
Application Note:	Sheep 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. Sheep 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 20 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

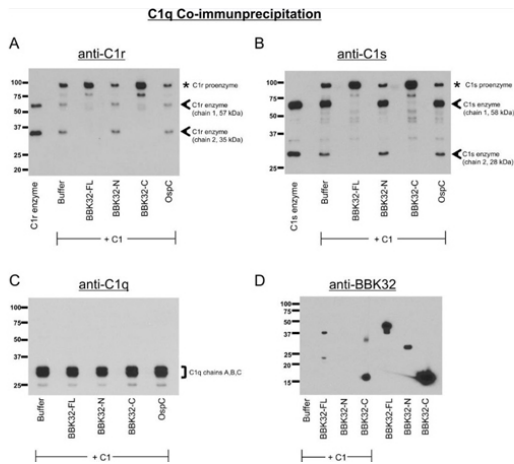
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1000X (see application note, titrated reagent enough for 20 blots) by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	Proclin is added as an antimicrobial agent.
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



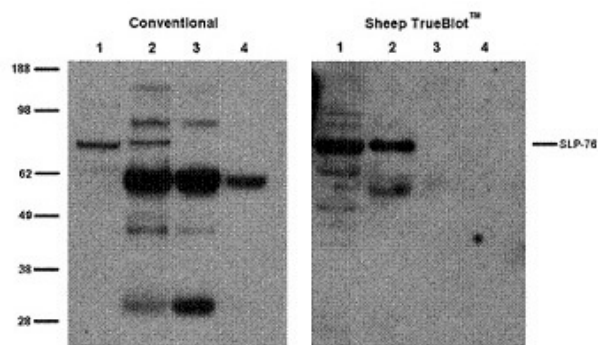
Immunoprecipitation

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 μ M 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 contain 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

Sheep TrueBlot® IP / Western Blot: Jurkat cell lysate (500 µg) was incubated with 2 µg of sheep anti-SLP76 and immunoprecipitated using Protein G. 20 µg Jurkat lysate (lane 1), immunoprecipitate (lane 2) and 1.0 µg and 0.1.0 µg reduced anti-SLP76 (lanes 3 and 4) were subjected to electrophoresis, transferred to a PVDF membrane, and immunoblotted with anti-SLP76 using conventional HRP-conjugated anti-sheep polyclonal antibody (left panel) and Sheep TrueBlot® ULTRA: Anti-Sheep IgG HRP (right panel).

References

- Garcia et al. *Borrelia burgdorferi* BBK32 Inhibits the Classical Pathway by Blocking Activation of the C1 Complement Complex. *PLOS Pathogens* (2016)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.