

Datasheet for 88-8887-383**TrueBlot® Immunoprecipitation and Western Blot Kit for DYKDDDDK (FLAG®) Epitope Tag****Overview**

Description:	TrueBlot® Immunoprecipitation and Western Blot Kit for DYKDDDDK (FLAG®) Epitope Tag - 88-8887-383
Item No.:	88-8887-383
Size:	1 Kit
Applications:	IP, WB
Reactivity:	Mouse, FLAG-Tag

Product Details**Background:**

The FLAG® epitope is a small but highly immunogenic peptide DYKDDDDK (N-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-C) which allows fusion proteins to retain their original conformation and function. The hydrophilic character of this sequence increases the likelihood that it will be located on the surface of FLAG®-tagged recombinant proteins for reaction with anti-DYKDDDDK (FLAG®) antibodies. The Anti-DYKDDDDK (FLAG®) antibody included in this Western blot kit detects FLAG®-tagged recombinant proteins fused with FLAG® tag either at the amino-terminus or carboxy-terminus of the protein. The TrueBlot® Immunoprecipitation and Western Blot Kit for DYKDDDDK (FLAG®) Epitope Tag contains the critical supporting reagents, buffers, and substrates for immunoprecipitation and Western blotting of samples containing the DYKDDDDK (FLAG®) Epitope Tag using TrueBlot monoclonal secondary antibody in conjunction with Rockland's Antibody for the detection of FLAG™ conjugated proteins (MOUSE) Monoclonal Antibody (see application notes). Mouse IgG TrueBlot® ULTRA is the unique horseradish peroxidase conjugated Anti-Mouse IgG monoclonal secondary antibody 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 conventional HRP Anti-Mouse IgG immunoblotting secondary antibody. 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/immunoblot applications. Applications include studies examining post-translational modification (e.g., phosphorylation or acetylation) or protein-protein interactions.

Synonyms:	HRP, TrueBlot, HRP TrueBlot ULTRA, Peroxidase TrueBlot, TrueBlot for IP/WB, TrueBlot for immunoprecipitation, TrueBlot for western blotting, Flag Western Blot, Western Blotting Kit, Chemiluminescent Flag Kit, Immunoblotting Kit, FLAG®, HRP Anti FLAG, DDK, FLAG, FLAG antibody, anti-Flag, anti-DDK, DDK antibody
Conjugate:	Peroxidase (HRP) ULTRA
Clonality:	Monoclonal
Clone ID:	29E4.G7
Format:	IgG
Detection Kit Type:	Immunoprecipitation Kit

Target Details

Reactivity:	Mouse, FLAG-Tag
Purity/Specificity:	This TrueBlot® Immunoprecipitation and Western Blot Kit for DYKDDDDK (FLAG®) Epitope Tag allows for the detection of FLAG®-tagged recombinant protein present in cell lysates provided by the user. Mouse TrueBlot® ULTRA 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-Mouse Serum. Reactivity is observed against native Mouse IgG by both Western blot and ELISA.
Relevant Links:	• FLAG TrueBlot Protocol • IP and WB Kit for (FLAG®) Tag SDS

Application Details

Tested Applications:	IP, WB
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Application Note:

Mouse IgG TrueBlot® ULTRA 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). Mouse IgG TrueBlot® ULTRA is a HRP-conjugated monoclonal secondary antibody reacting with mouse IgGs for optimal signal detection in immunoprecipitation/immunoblot experiments. 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. TrueBlot® IP and WB Kit for DYKDDDDK (FLAG®) Epitope Tag 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. Anti-Mouse Ig IP Beads: 2.5 mL Binds 0.4 mg Ig/mL beads; 8. Antibody for the detection of FLAG™ conjugated proteins (MOUSE) Monoclonal Antibody: 100 µg; 9. Western Blot Incubation Tray. Special Notes: Upon initial use of the IP beads, we recommend that the vial be inverted several times to get the beads into suspension. We recommend using a large bore pipet to pipet up the liquid for use. For storage of the opened vial of beads, we recommend that the vial cap be sealed with parafilm to help prevent evaporation of the buffer. 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.

Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IP:	1-10 µg / 10 ⁷ cells/1 mL lysate
WB:	1:1000

Formulation

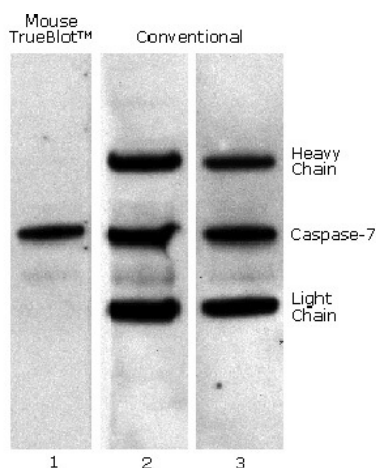
Physical State:	Liquid (sterile filtered)
Concentration:	1 Kit
Buffer:	0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	Wash buffers MUST NOT contain SODIUM AZIDE or other inhibitors of peroxidase activity!
Stabilizer:	0.1 mg/ml Bovine Serum Albumin (BSA) - IgG and Protease free, 50% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store Kit at 2-8 °C, except Mouse TrueBlot® ULTRA and Antibody for the detection of FLAG™ conjugated proteins (MOUSE) Monoclonal Antibody, which should be stored 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



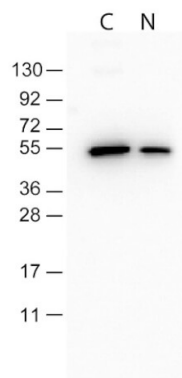
Western Blot

Mouse TrueBlot® IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1×10^7 Jurkat cells/ml with 5 ug mouse anti-human Caspase 7. Precipitate from 1×10^6 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.

Kit Box

This product is assembled as a kit. See attached protocol or CofA for further details.





Western Blot

Monoclonal Antibody to detect FLAG™ conjugated proteins detects both C terminal linked and N terminal linked FLAG™ tagged recombinant proteins by western blot.



Immunoprecipitation

Immunoprecipitation of VHH-EGFR-FLAG single chain antibody. 500 ug of HeLa Whole Cell Lysate (p/n W09-000-364) spiked with 1 ug of VHH-EGFR-FLAG single chain antibody was immunoprecipitated with 1 ug of Mouse anti-FLAG® Monoclonal Antibody (p/n 200-301-383) overnight at 4°C. Western Blotting of Immunoprecipitation product followed. Lane 1 - VHH-EGFR-FLAG Immunoprecipitation. Lane 2 - VHH-EGFR-FLAG Pre-Cleared Lysate (prior to immunoprecipitation). Load: 2 uL per lane. Primary Antibody: Mouse anti-FLAG® Monoclonal Antibody at 1:1,000 o/n at 4°C. Secondary Antibody: Mouse Trueblot® ULTRA: Anti-Mouse Ig HRP at 1:2,000 for 1 HR at RT. Block: MB-070 for 2 HR at RT. Predicted/Observed size: 11 kDa, 11 kDa for VHH-EGFR-FLAG single chain antibody.

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.