Datasheet for ABIN6809839

TrueBlot® Immunoprecipitation and Western Blot Kit for DYKDDDDK (FLAG®) Epitope Tag

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Overview

Quantity:	1 kit
Application:	Immunoprecipitation (IP), Western Blotting (WB)

Product Details

Purpose:	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
Brand:	TrueBlot®
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.
Characteristics:	The FLAG® epitope is a small but highly immunogenic peptide DYKDDDDK (N-Asp-Tyr-Lys-Asp-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

Product Details

	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 imonoclonal 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.
Components:	TrueBlot® IP and WB Kit for 6X HIS Epitope Tag Components: 1. Mouse IgG TrueBlot ULTRA: 50 µL ABIN1589976 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. Anti-6X HIS (MOUSE) Monoclonal Antibody: 100 µg 9. Western Blot Incubation Tray.

Application Details

Application Notes:	Western Blot : 1:1000 ImmunoPrecipitation: 1-10 µg / 10^7 cells/1 mL lysate
Comment:	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 an HRP-conjugated monoclonal secondary antibody reacting with mouse IgGs for optimal signal detection in immunoprecipitation/immunoblotting experiments. Note that there are three key procedural considerations:

Application Details

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.

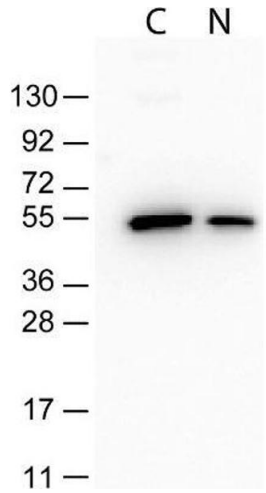
Special Notes: Upon initial use of the IP beads, we recommend that the vial be inverted several times to get the beads into suspension. We recommend using a large bore pipet to pipet up the liquid for use. For storage of the opened vial of beads, we recommend that the vial cap be sealed with parafilm 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.

Restrictions:	For Research Use only

Handling

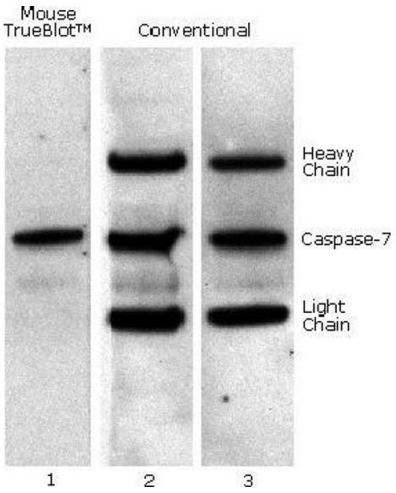
Buffer:	Buffer: 0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: 0.1 mg/mL Bovine Serum Albumin (BSA) - IgG and Protease free, 50 % (v/v) Glycerol Wash buffers MUST NOT contain SODIUM AZIDE or other inhibitors of peroxidase activity!
Storage:	4 °C
Storage Comment:	Store Kit at 2-8 °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.

Images



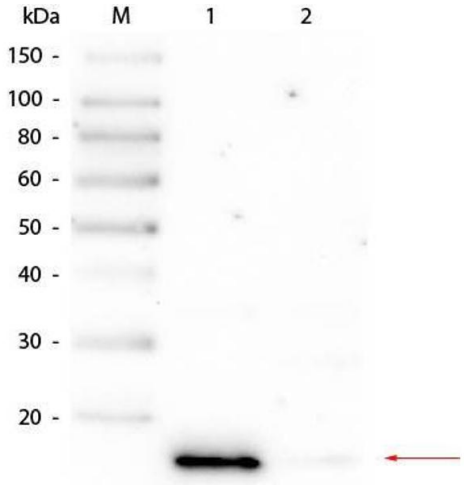
Western Blotting

Image 1. Western Blot-Monoclonal Antibody to detect conjugated proteins Monoclonal Antibody to detect conjugated proteins detects both C terminal linked and N terminal linked tagged recombinant proteins by western blot.



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. Immunoprecipitation of VHH-EGFR-FLAG single chain antibody. 500 ug of Hela Whole Cell Lysate spiked with 1 ug of VHH-EGFR-FLAG single chain antibody was immunoprecipitated with 1 ug of Mouse anti-FLAG® Monoclonal Antibody 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 ULTRA: Anti-Mouse Ig HRP at 1:2,000 for 1 HR at RT. Block: ABIN925618 for 2 HR at RT. Predicted/Observed size: 11 kDa, 11 kDa for VHH-EGFR-FLAG single chain antibody.