Datasheet for ABIN6809838

TrueBlot® Immunoprecipitation and Western Blot Kit for 6X HIS Epitope Tag

2 Images

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Overview

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

Product Details

Purpose:	TrueBlot® Immunoprecipitation and Western Blot Kit for 6X HIS Epitope Tag allows for the immunoprecipitation and detection of 6X HIS-tagged recombinant proteins present in cell lysates
Brand:	TrueBlot®
Specificity:	This TrueBlot® Immunoprecipitation and Western Blot Kit for 6X HIS Epitope Tag allows for the detection of 6X HIS-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:	TrueBlot® Immunoprecipitation and Western Blot Kit for 6X HIS Epitope Tag allows for the immunoprecipitation and detection of 6X HIS-tagged recombinant proteins present in cell lysates provided by the user. After protein separation by SDS-PAGE and transfer, the membrane is probed with monoclonal Anti-6X HIS. Detection of the membrane bound antibody-antigen complex is achieved by the addition of Mouse IgG TrueBlot® ULTRA HRP. The TrueBlot® Immunoprecipitation and Western Blot Kit for 6X HIS Epitope Tag contains the critical supporting reagents, buffers, and substrates for immunoprecipitation and Western blotting of samples containing a 6X HIS Epitope Tag using TrueBlot monoclonal secondary antibody in conjunction with Rockland's Anti-6X HIS (MOUSE) Monoclonal Antibody. Mouse IgG TrueBlot®

Product Details

	ULTRA is the unique horseradish peroxidase conjugated Anti-Mouse IgG monoclonal secondary antibody which enables detection of immunoblotted target protein bands, without hindrance from 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/immunoblotting 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: 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.

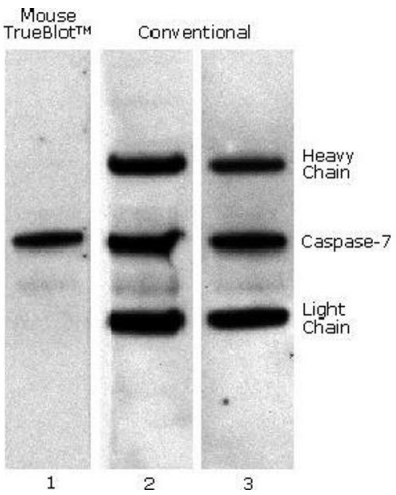
Application Details

	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

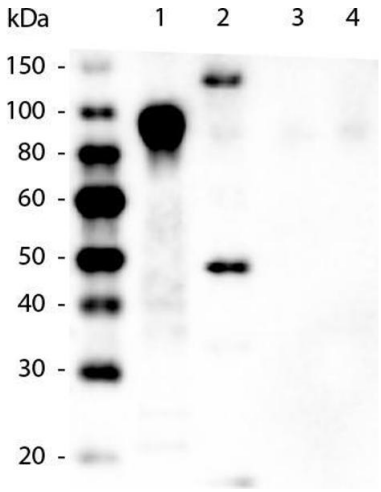
Buffer:	Buffer: 0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: 0.1 mg/mL Bovine Serum Albumin (BSA) - IgG and Protease free, 50 % (v/v) Glycerol Wash buffers MUST NOT contain SODIUM AZIDE or other inhibitors of peroxidase activity!
Storage:	4 °C
Storage Comment:	Store Kit at 2-8 °C, except Mouse TrueBlot® ULTRA and Anti-6X HIS (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. 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 2. Western Blot of Mouse anti-6X HIS Tag Antibody. Lane 1: 100ng Purified histidine-tagged recombinant protein. Lane 2: 200ng E. coli cell lysate containing histidine-tagged expression construct. Lane 3: 100ng Purified GST-tagged recombinant protein. Lane 4: 100ng Purified FLAG-tagged recombinant protein. Primary antibody: Mouse anti-6X HIS Tag antibody at 1:5,000 overnight at 4°C. Secondary antibody: Peroxidase mouse secondary antibody at 1:20,000 for 30 min at RT. Block: 5% BLOTTO for 1 hr at RT.