

Datasheet for ABIN6809837

TrueBlot® Immunoprecipitation and Western Blot Kit for GFP Epitope Tag[Go to Product page](#)**3** Images

Overview

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

Product Details

Purpose:	TrueBlot® Immunoprecipitation and Western Blot Kit for GFP Epitope Tag allows for the immunoprecipitation and detection of GFP-tagged recombinant proteins present in cell lysates
Brand:	TrueBlot®
Specificity:	<p>This TrueBlot® Immunoprecipitation and Western Blot Kit for GFP Epitope Tag allows for the detection of GFP-tagged recombinant protein present in cell lysates provided by the user.</p> <p>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.</p>
Characteristics:	<p>TrueBlot® Immunoprecipitation and Western Blot Kit for GFP Epitope Tag allows for the immunoprecipitation and detection of GFP-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-GFP. 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 GFP Epitope Tag contains the critical supporting reagents, buffers, and substrates for immunoprecipitation and western blot containing a GFP Epitope Tag using TrueBlot secondary antibody immunoblotting reagents in conjunction with Rockland's Anti-GFP (MOUSE) Monoclonal Antibody. Mouse IgG TrueBlot® ULTRA is the unique horseradish peroxidase conjugated Anti-Mouse IgG monoclonal secondary antibody</p>

Product Details

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 secondary antibody. It is easy to generate publication-quality IP/WB data with Mouse IgG TrueBlot® ULTRA.

Components:

TrueBlot® IP and WB Kit for GFP Epitope Components:

- Mouse IgG TrueBlot ULTRA: 50 µL ABIN1589976
- TrueBlot Enhancer Solution: 25 mL
- TrueBlot Blocker: 10 g
- TrueBlot Assay Buffer: 30 mL 20X
- TrueBlot Substrate A: 12.5 mL
- TrueBlot Substrate B: 12.5 mL
- Anti-Mouse Ig IP Beads: 2.5 mL Binds 0.4 mg Ig/mL beads
- Anti-GFP (MOUSE) Monoclonal Antibody: 100 µg
- Western Blot Incubation Tray.

Application Details

Application Notes:

Western Blot : 1:1000

ImmunoPrecipitation: 1-10 µg / 10⁷ 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. 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.

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

Application Details

sealed with parafilm to help prevent evaporation of the buffer.

Restrictions: For Research Use only

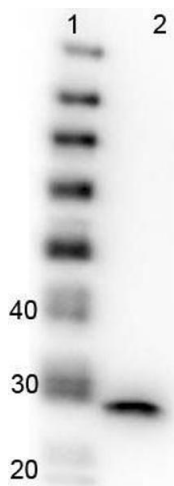
Handling

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

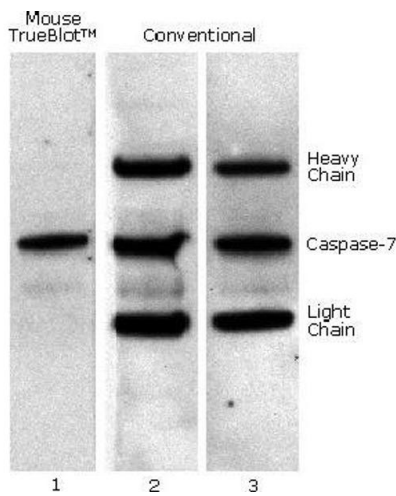
Storage Comment: Store Kit at 2-8 °C, except Mouse TrueBlot® ULTRA and Anti-GFP (MOUSE) Monoclonal Antibody, which should be stored at -20 °C. This product is guaranteed for 6 months upon receipt, when handled and stored as instructed.

Images



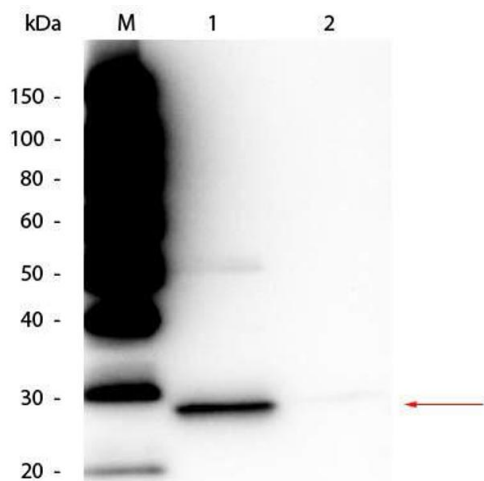
Western Blotting

Image 1. Western Blot of GFP Western Blot of Mouse anti-GFP antibody. Lane 1: Molecular Weight Markers. Lane 2: GFP. Load: 10ng per lane. Primary antibody: Ms Anti-GFP antibody at 1:1000 for overnight at 4°C. Secondary antibody: Mouse HRP secondary antibody at 1:40,000 for 30 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 27 kDa, 27 kDa for epitope tag GFP. Other band(s): None.



Western Blotting

Image 2. Mouse IP / Western Blot: Caspase 7 was immunoprecipitated from 0.5 ml of 1x10⁷ Jurkat cells/ml with 5 ug mouse anti-human Caspase 7. Precipitate from 1x10⁶ 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 recombinant GFP. 500 ug of HeLa Whole Cell Lysate spiked with 1 ug of recombinant GFP was immunoprecipitated with 2 ug of Mouse anti-GFP Monoclonal Antibody overnight at 4°C. Western Blotting of Immunoprecipitation product followed. Lane 1 - recombinant GFP Immunoprecipitation. Lane 2 - recombinant GFP Pre-Cleared Lysate (prior to immunoprecipitation). Load: 2 uL per lane. Primary Antibody: Mouse anti-GFP 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: 28 kDa, 28 kDa for recombinant GFP.