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Datasheet for 00-8811-25

TrueBlot® Anti-Mouse Ig IP Agarose Beads

Overview

Description:	TrueBlot® Anti-Mouse Ig IP Agarose Beads - 00-8811-25
Item No.:	00-8811-25
Size:	2.5 mL
Applications:	IP, SDS-PAGE, WB, Biochemical Assay
Reactivity:	Mouse
Host Species:	Goat

Product Details

Background:	TrueBlot® Anti-Mouse Ig IP Agarose Beads are a suspension of activated agarose beads coupled with goat Anti-mouse IgG. It is suitable for precipitation of mouse IgGs used as the primary antibodies in immunoprecipitation assays. The beads are in suspension and will settle upon storage. Prior to use, mix the vial gently (do not vortex) to ensure delivery of proper bead volume.
Synonyms:	Anti-Mouse immunoglobulin Gamma, Agarose-conjugated IgG, Gt-a-Ms IgG, Goat anti-Mouse IgG, TrueBlot, TrueBlot for immunoprecipitation, IP Agarose beads for TrueBlot.
Host Species:	Goat
Conjugate:	Agarose ULTRA
Clonality:	Polyclonal
Format:	IgG
Detection Kit Type:	Immunoprecipitation Kit

Target Details

Reactivity:	Mouse
Purity/Specificity:	TrueBlot® Anti-Mouse Ig IP Agarose Beads has been tested in SDS-Page, immunoprecipitation, and western blot.
Relevant Links:	• TrueBlot IP Protocol

Application Details

Tested Applications:	IP, SDS-PAGE, WB
Suggested Applications:	Biochemical Assay (Based on references)
Application Note:	Upon initial use of this product, 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, we recommend that the vial cap be sealed with parafilm to help prevent evaporation of the buffer. Procedure: Preparation of Immunoprecipitated Sample for SDS-PAGE: 1. Preclear cell lysate: Add 50 µL of Anti-Mouse IgG Beads and 500 µL of cell lysate sample to a microcentrifuge tube and incubate on ice for 30 minutes. Spin at 10,000xg for 3 minutes and transfer the supernatant to a new microcentrifuge tube. 2. Immunoprecipitation: Add 5 µg of primary antibody to the microcentrifuge tube containing the precleared lysate. Incubate on ice for 1 hour. Add 50 µL of Anti-Mouse IgG Beads. Incubate for 1 hour on a rocking platform. Spin the microcentrifuge tube at 10,000xg for 1 minute. Remove supernatant completely and wash the (pelleted) beads 3 times with 500 µL of Lysis Buffer (50mM Tris HCl, pH 8.0; 150mM NaCl; 1% NP-40). 3. Prepare sample for SDS-PAGE: After the last wash, aspirate supernatant, and add 50 µL Laemmli Buffer (with 50 mM DTT or 2% β-mercaptoethanol, final) to bead pellet. Vortex and heat to 90-100 °C for 10 minutes. Spin at 10,000xg for 3 minutes, collect supernatant, and load onto the gel. Avoid loading Anti-Mouse Ig Beads. Note: The supernatant can be stored at -20 °C for future use. After thawing, add fresh dithiothreitol and heat as above. Centrifuge the sample at 10,000xg for 1 minute in a microcentrifuge tube to pellet any Anti-Mouse Ig Beads and immediately transfer an aliquot of the supernatant to gel wells.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IP:	TrueBlot anti-Mouse Ig IP Beads (binds 0.4 mg Ig/ml beads) have been reported for use in IP
WB:	Use with Mouse TrueBlot® (cat # 18-8817-33)

Formulation

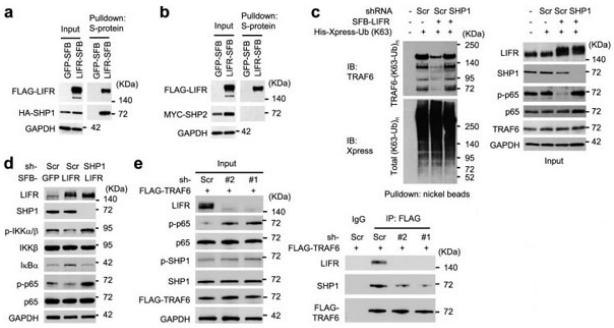
Physical State:	Suspension of agarose beads
Concentration:	14.4 mg/cc antibody per cc agarose
Buffer:	0.01 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.09% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

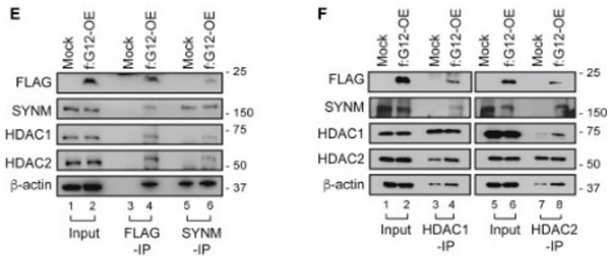
Shipping Condition:	Wet Ice

Storage Condition:	Store vial at 4 °C prior to opening. DO NOT FREEZE.
Expiration:	Expiration date is six (6) months from date of receipt.

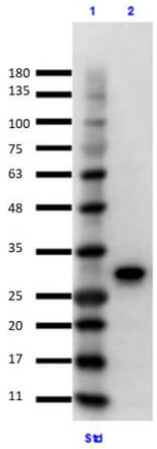
Images



Immunoprecipitation
Loss of LIFR activates NF-κB signaling through SHP1, leading to upregulation of LCN2. (a) HEK293T cells were transfected with HA-FLAG-SHP1 and SFB-tagged GFP or LIFR. LIFR-SFB protein was pulled down with S-protein beads, followed by immunoblotting with antibodies against FLAG and HA. (b) HEK293T cells were transfected with MYC-SHP2 and SFB-tagged GFP or LIFR. LIFR-SFB protein was pulled down with S-protein beads, followed by immunoblotting with antibodies against FLAG and MYC. (c) HEK293T SFB-GFP and SFB-LIFR stable cell lines were infected with the scrambled (Scr) or sh-SHP1 lentivirus, followed by transfection with a K63-specific mutant of His-Xpress-ubiquitin (Ub). 48 h later, cells were subjected to pulldown with nickel beads and immunoblotting with antibodies against TRAF6 and Xpress. (d) Control and LIFR-overexpressing PLC/PRF/5 cells were transduced with SHP1 shRNA and immunoblotted with the indicated antibodies. (e) Control (Scr) and LIFR-knockdown HEK293T cells were transfected with FLAG-TRAF6. 48 h later, cells were immunoprecipitated with a FLAG-specific antibody and immunoblotted with antibodies against LIFR, SHP1, and FLAG. Fig. 4. PMID: 34921145.



Immunoprecipitation
GAGE12 modulates H3K56Ac through attenuation of HDAC activity.
(E) Interaction of FLAG-GAGE12, SYNM, HDAC1/2, and β-actin in total nuclear lysates of mock and f:G12-OE cells. In FLAG immunoprecipitates, SYNM, HDAC1/2, and β-actin are only pulled down in the presence of FLAG-GAGE12 (lanes 3 and 4). In SYMN immunoprecipitates, SYNM only pulled down HDAC1 and HDAC2 in the presence of FLAG-GAGE12. SYNM interacts with β-actin, even in the absence of GAGE12 (lanes 5 versus 6). (F) Increased HDAC1-HDAC2-β-actin interaction in the presence of GAGE12. In HDAC1 and HDAC2 immunoprecipitates, increased β-actin was found in the pull-down lysates only in the presence of GAGE12. Figure 6. PMID: 34469741.



Western Blot
Immunoprecipitation/Western Blot using GFP Protein. Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 2: GFP Input (p/n 000-001-215) Reduced [10µL]. Primary IP Antibody: Mouse Anti-GFP (p/n 600-301-215) at 10µg overnight at 2-8°C. Secondary Antibody: TrueBlot Anti-Mouse Ig IP Agarose Beads (p/n 00-8811-25) at 500µg for 1hr at RT. Buffer: BlockOut Buffer (p/n MB-073) for 30 mins at RT. Exposure: 7 sec.

References

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