ANTIBODIES ONLINE

Datasheet for ABIN1690141

anti-CTNNB1 antibody (Magnetic Particles)

1 Validation

Go to Product page

Overview

Quantity:	100 μg
Target:	CTNNB1
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This CTNNB1 antibody is conjugated to Magnetic Particles
Application:	Immunoprecipitation (IP)

Product Details

Target Details

Target:	CTNNB1
Alternative Name:	CTNNB1 / Beta Catenin (CTNNB1 Products)
Pathways:	WNT Signaling, Intracellular Steroid Hormone Receptor Signaling Pathway, Peptide Hormone Metabolism, Regulation of Muscle Cell Differentiation, Cell-Cell Junction Organization, Tube Formation, Maintenance of Protein Location, Signaling Events mediated by VEGFR1 and VEGFR2

Application Details

Restrictions:	For Research Use only

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Handling

Storage: 4 °C

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Successfully validated (Immunoprecipitation (IP))

by Institute of Musculoskeletal Sciences, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford

Report Number: 100087

Date: Mar 13 2017

Target:	CTNNB1
Lot Number:	416
Method validated:	Immunoprecipitation (IP)
Positive Control:	Colo205 human colorectal cell line
Notes:	ABIN1690141 (CTNNB1 antibody conjugated to magnetic particles) was found to be suitable for IP of beta-catenin from lysates of Colo205 cells.
Primary Antibody:	ABIN1690141

Protocol:

- Cell lysates:
  - Prepare detergent-free lysates of Colo205 cells on ice in low detergent 'soft' elution buffer (150mM NaCl, 50mM Tris-HCl, 0.02% Tween-20, pH8.0; see Antrobus R and Borner GH, (2011) PLoS One) supplemented with Halt Protease and Phosphatase inhibitor cocktail (Thermo Fisher, 78440, lot RE234421) using a TissueRuptor (Qiagen).
  - Pellet insoluble debris by centrifugation.
  - Transfer the supernatant and quantitate the total protein concentration by CB-X Protein Assay (G Biosciences, 786-11X, lot 152710).
- Immunoprecipitation:
  - Dilute supernatant to 1mg/ml total protein.
  - Equilibrate CTNNB1 antibody magnetic particles (antibodies-online, ABIN1690141, lot 0416) with PBS and then wash them 3x with PBS to remove residual detergent.
  - Incubate 50µg CTNNB1 antibody magnetic particles (antibodies-online, ABIN1690141, lot 0416) with 1ml of Colo205 lysate at 1mg/ml total protein ON at 4°C on an end-over-end rotator.
  - Recover the magnetic beads on a Dynabead rack (ThermoFisher). Store the removed supernatant at -80°C for the subsequent immunoblot.
  - Wash beads 5x with 1ml ice cold PBS supplemented with Halt cocktail. Store each wash fraction at -80°C for the subsequent immunoblot.
  - Incubate immunoprecipitated proteins with 50µl 'soft' reduced detergent elution buffer (0.2% (w/v) SDS, 0.1% (v/v) Tween-20, 50mM Tris-HCl pH8.0) suitable for downstream mass spectrometric analysis.
- Immunoblot:
  - Separate proteins on a 10% SDS-PAGE gel using a Mini-PROTEAN 3 cell electrophoresis

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tank (Bio-Rad, 165-3301/165-3302) at 200 V for 90min at RT and Tris-Glycine-SDS running buffer.
    - Transfer proteins to PVDF immobilon-P membrane (Millipore, IPVH00010, R6kA7917F) using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, 170-3930/170-3935) at 30V, 90mA, ON at 4°C in Tris-Glycine transfer buffer.
    - Incubate with a primary mouse purified mouse-anti-beta-catenin antibody (BD Biosciences, 610154, LOT 5113978) diluted 1:1000x in TBST (TBS, 0.1% (v/v) Tween-20) with 5% non-fat milk for 2h at RT.
    - Wash membrane 3x 5min with TBST.
    - Incubate with HRP-conjugated polyclonal goat anti-mouse secondary antibody (Dako, P0447, lot 00082889) diluted 1:2000 in TBST with 5% non-fat milk for 1h at RT.
    - Wash membrane 3x 5min with TBST.
    - Visualized immunoreactive bands by autoradiography. Incubate membrane for 1min at RT with 3ml of ECL Western Blotting Substrate (Promega, W1001, lot 0000193677) and expose to Hyperfilm ECL (Amersham, product 28906837, lot 64701) for 5min in the dark.
- Mass spectrometric analysis:
    - Extract peptides from the gel band and submit to a tryptic digest with Sequencing Grade Modified Trypsin (Promega, V5111).
    - Run tryptic fragments on a Q Exactive (QEX) Hybrid Quadrupole-Orbitrap Mass Spectrometer (ThermoFisher).
    - Identify proteins fragments by performing an MS/MS Ion search with MASCOT (Matrix Science) against the UPR HomoSapiens 20160706 database (92,578 sequences; 36,833,215 residues).

Experimental Notes:

- ABIN1690141 proved to be be susceptible to detergents as it was evidenced by the higher efficacy of the lower detergent 'soft' elution buffer compared to RIPA buffer with PBS.
- The IP can be improved through the use of detergent-free protein lysate preparations and by washing with PBS rather than RIPA, as strong detergents can strip beta-catenin from the antibodies on the beads. The 'soft', low detergent elution buffer formulated by Antrobus and Borner is recommended where the eluted protein is to be used for mass spectrometry.
- Assuming that the lysate for the IP came from the same source, so there is no danger of cross-contamination, the beads can be re-used approximately four times.

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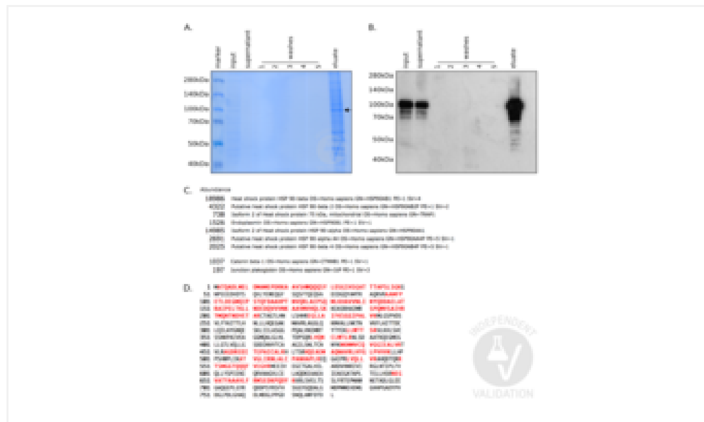

Validation image no. 1 for anti-Catenin (Cadherin-Associated Protein), beta 1, 88kDa (CTNNB1) antibody (Magnetic Particles) (ABIN1690141)

A. SDS-PAGE of protein fractions from immunoprecipitation of beta-catenin with ABIN1690141. Gel was stained with GelCode (ThermoFisher), a Coomassie blue dye. The prominent band at 100kDa (arrow) was excised and sent for mass spectrometry. B. Immunoblot of the same protein fractions as in A. Note the strong beta-catenin immunoreactivity of the eluate band corresponding to the 100kDa band in A. C. MASCOT output edited to show the essential features of the analysis. Peptides from beta-catenin are detectable in a tryptic digest of proteins extracted from the Coomassie band shown in A. but peptides from heat shock protein 90 (Hsp90) are far more abundant. D. MASCOT output edited to show the essential features of the analysis. Protein sequence coverage of beta-catenin after a tryptic digest is 39%. However, the detectable matched peptides (bold red) capture the majority of sites known to be modified post-translationally in beta-catenin.

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