abcam

Product datasheet

Anti-SKP2 antibody [EPR3305(2)] ab183039





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Overview

Product name Anti-SKP2 antibody [EPR3305(2)]

Description Rabbit monoclonal [EPR3305(2)] to SKP2

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: C6, NIH/3T3, F9, A549, MDA-MB-231, Jurkat, MCF7, HepG2, and NCCIT cell lysates. ICC:

293T cells

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply - Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR3305(2)

Isotype ΙgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab183039 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/70. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★ ☆☆ <u>(1)</u>	1/100.
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/200. Detects a band of approximately 48 kDa (predicted molecular weight: 48 kDa).

Target

Function

Substrate recognition component of a SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins involved in cell cycle progression, signal transduction and transcription. Specifically recognizes phosphorylated CDKN1B/p27kip and is involved in regulation of G1/S transition. Degradation of CDKN1B/p27kip also requires CKS1. Recognizes target proteins ORC1, CDT1, RBL2, MLL, CDK9, RAG2, FOXO1A, UBP43, and probably MYC, TOB1 and TAL1. Degradation of TAL1 also requires STUB1. Recognizes CDKN1A in association with CCNE1 or CCNE2 and CDK2.

Pathway

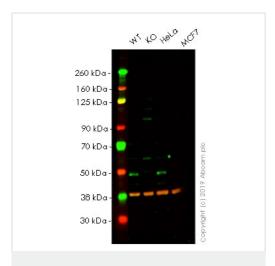
Protein modification; protein ubiquitination.

Sequence similarities

Contains 1 F-box domain.

Contains 9 LRR (leucine-rich) repeats.

Images



Western blot - Anti-SKP2 antibody [EPR3305(2)] (ab183039)

All lanes : Anti-SKP2 antibody [EPR3305(2)] (ab183039) at 1/200 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: SKP2 knockout HAP1 whole cell lysate

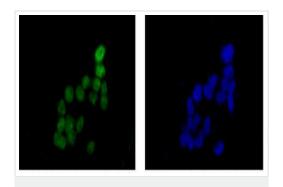
Lane 3 : HeLa whole cell lysate
Lane 4 : MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 48 kDa

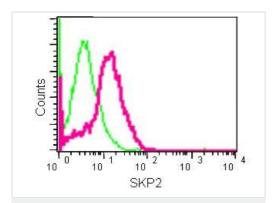
Lanes 1 - 4: Merged signal (red and green). Green - ab183039 observed at 52 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab183039 was shown to recognize in wild-type HAP1 cells as signal was lost at the expected MW in SKP2 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and SKP2 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab183039 and ab8245 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/200 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



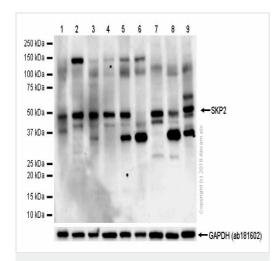
Immunocytochemistry/ Immunofluorescence - Anti-SKP2 antibody [EPR3305(2)] (ab183039)

Immunofluorescenct analysis of 4% paraformaldehyde fixed 293T cells labeling SKP2 with ab183039 at 1/100 followed by Goat anti rabbit lgG(Alexa Fluor® 488) at 1/200 (green). Cells were counter stained with Dapi (blue).



Flow Cytometry (Intracellular) - Anti-SKP2 antibody [EPR3305(2)] (ab183039)

Intracellular flow cytometric analysis of 2% paraformaldehyde fixedHeLa cells labeling SKP2 with ab183039 at 1/70 followed byGoat anti rabbit lgG (FITC) at 1/150.Rabbit monoclonal lgG was used aslsotype control.



Western blot - Anti-SKP2 antibody [EPR3305(2)] (ab183039)

All lanes : Anti-SKP2 antibody [EPR3305(2)] (ab183039) at 1/200 dilution

Lane 1 : NCCIT (Human pluripotent embryonic carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 2: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 3 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 4: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates with 5% NFDM/TBST

Lane 5 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 6: A549 (Human lung carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 7: F9 (Mouse embryonal carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 8 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

with 5% NFDM/TBST

Lane 9 : C6 (Rat glial tumor glial cell) whole cell lysate with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

Secondary

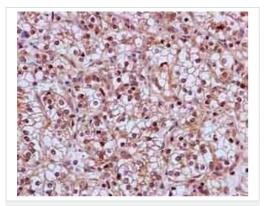
All lanes : Goat Anti-Rabbit lgG H&L (HRP) ($\underline{ab97051}$) at 1/20000

dilution

Predicted band size: 48 kDa

Exposure time: 180 seconds

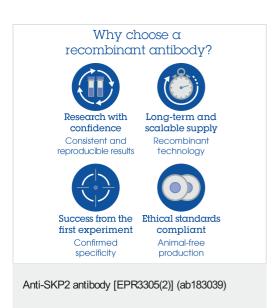
We recommend to increase the amount of samples or decrease antibody dilution to get clear bands.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SKP2 antibody
[EPR3305(2)] (ab183039)

immunohistochemical analysis of paraffin embedded Human kidney clear cell carcinoma tissue labeling SKP2 with ab183039 at 1/50 followed by secondary staining with Ready to use HRP Polymer for Rabbit IgG and counterstained with Hematoxylin.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



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