abcam

Product datasheet

Anti-AKT2 antibody [4H7] ab175354

16 References 9 Images

Overview

Product name	Anti-AKT2 antibody [4H7]
Description	Mouse monoclonal [4H7] to AKT2
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, ChIP, IP, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey **Predicted to work with:** Non human primates
Immunogen	Recombinant full length protein corresponding to Human AKT2 aa 1-481. (NP_001617) produced in HEK293T cell. Sequence: MNEVSVIKEGWLHKRGEYIKTWRPRYFLLKSDGSFIGYKE RPEAPDQTLP PLNNFSVAECQLMKTERPRPNTFVIRCLQWTTVIERTFHV DSPDEREEWM RAIQMVANSLKQRAPGEDPMDYKCGSPSDSSTTEEMEV AVSKARAKVTMN DFDYLKLLGKGTFGKVILVREKATGRYYAMKILRKEVIIAKD EVAHTVTE SRVLQNTRHPFLTALKYAFQTHDRLCFVMEYANGGELFFH LSRERVFTEE RARFYGAEIVSALEYLHSRDVVYRDIKLENLMLDKDGHIKIT DFGLCKEG ISDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVV MYEMMCGRL PFYNQDHERLFELILMEEIRFPRTLSPEAKSLLAGLLKKDP KQRLGGGPS DAKEVMEHRFFLSINWQDVVQKKLLPPFKPQVTSEVDTR YFDDEFTAQSI TITPPDRYDSLGLLELDQRTHFPQFSYSASIRE Database link: **P31751** Run BLAST with Run BLAST with
Positive control	MCF7, HeLa, HepG2, A549, 293T, Jurkat, A431, U2OS, COS7, 3T3 L1 and NRK whole celll lysates; AKT2 transfected U2OS cells; Human Medulla Oblongata tissue; Human Esophageal cancer tissue.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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	Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 69% PBS, 30% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	4H7
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab175354 in the following tested applications.

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

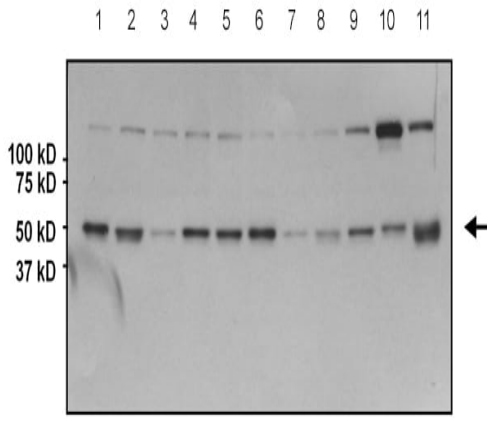
Application	Abreviews	Notes
ICC/IF		1/10 - 1/100.
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration. Use 2µg.
IHC-P		1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 55 kDa.

Target

Function	General protein kinase capable of phosphorylating several known proteins.
Tissue specificity	Expressed in all human cell types so far analyzed.
Sequence similarities	Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. RAC subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 PH domain.

	Contains 1 protein kinase domain.
Post-translational modifications	Phosphorylation on Thr-309 and Ser-474 is required for full activity. Ubiquitinated; undergoes both 'Lys-48'- and 'Lys-63'-linked polyubiquitination. TRAF6-induced 'Lys-63'-linked AKT2 ubiquitination. When fully phosphorylated and translocated into the nucleus, undergoes 'Lys-48'-polyubiquitination catalyzed by TTC3, leading to its degradation by the proteasome.

Images



Western blot - Anti-AKT2 antibody [4H7] (ab175354)

All lanes : Anti-AKT2 antibody [4H7] (ab175354) at 1/1000 dilution

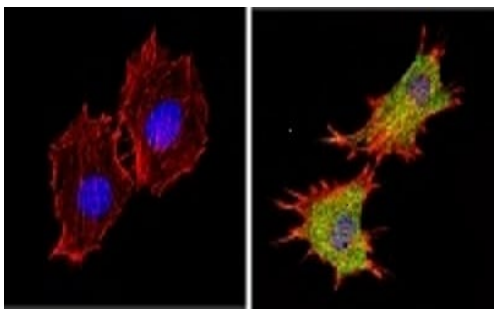
Lane 1 : MCF7 whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : HepG2 whole cell lysate
Lane 4 : A549 whole cell lysate
Lane 5 : 293T whole cell lysate
Lane 6 : Jurkat whole cell lysate
Lane 7 : A431 whole cell lysate
Lane 8 : U2OS whole cell lysate
Lane 9 : COS7 whole cell lysate
Lane 10 : 3T3 L1 whole cell lysate
Lane 11 : NRK whole cell lysate

Lysates/proteins at 25 µg per lane.

Secondary
All lanes : goat anti-mouse-HRP at 1/20000 dilution

Developed using the ECL technique.

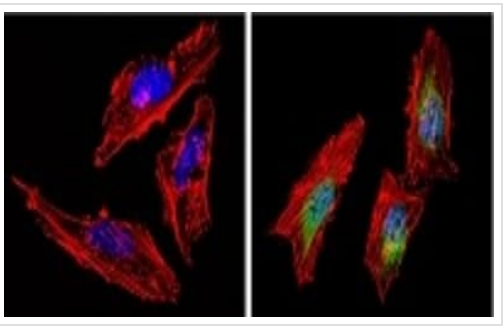
Predicted band size: 55 kDa



Immunocytochemistry/ Immunofluorescence - Anti-AKT2 antibody [4H7] (ab175354)

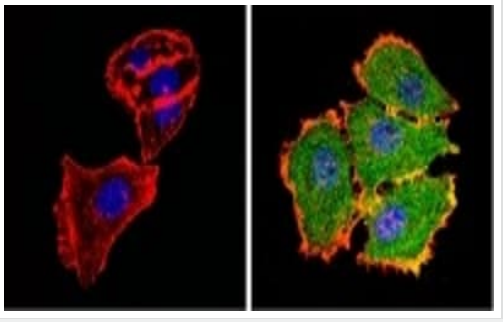
Immunofluorescent analysis of AKT2 (green) showing staining in the cytoplasm and nucleus of C2C12 cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with an AKT2 monoclonal antibody (ab175354) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with

Hoechst or DAPI. Images were taken at a magnification of 60x.



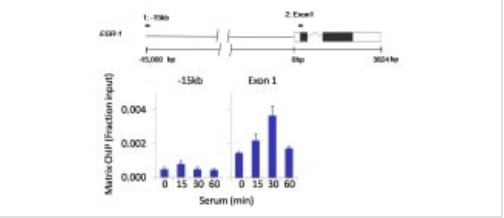
Immunocytochemistry/ Immunofluorescence - Anti-AKT2 antibody [4H7] (ab175354)

Immunofluorescent analysis of AKT2 (green) showing staining in the cytoplasm and nucleus of Hela cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with an AKT2 monoclonal antibody (ab175354) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



Immunocytochemistry/ Immunofluorescence - Anti-AKT2 antibody [4H7] (ab175354)

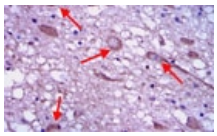
Immunofluorescent analysis of AKT2 (green) showing staining in the cytoplasm and nucleus of MCF-7 cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with an AKT2 monoclonal antibody (ab175354) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



ChIP - Anti-AKT2 antibody [4H7] (ab175354)

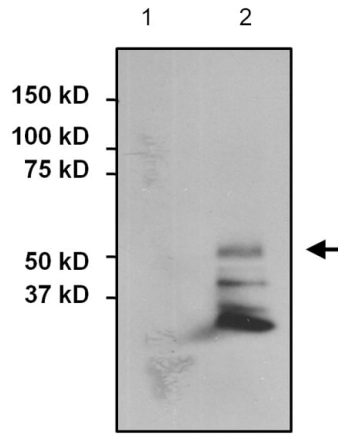
Chromatin immunoprecipitation analysis of Akt1 and Akt2 was performed using cross-linked chromatin from 1 x 10^6 HCT116 colon carcinoma cells treated with serum for 0, 15, 30, and 60 minutes. Immunoprecipitation was performed with 1.0ul/100ul well volume of an Atk1 monoclonal antibody and an Akt2 monoclonal antibody (ab175354). Chromatin aliquots from ~1 x 10^5 cells were used per ChIP pull-down. Quantitative PCR data were done in quadruplicate using 1ul of eluted DNA in 2ul SYBR real-time PCR reactions containing primers to amplify -15kb upstream of the Egr1 gene or exon-1 of Egr1. PCR calibration curves were generated for each primer pair from a dilution series of sheared total genomic DNA. Quantitation of immunoprecipitated chromatin is presented as signal relative to the total amount of input chromatin. Results represent the mean +/- SEM for three experiments. A schematic representation of the Egr-1 locus is shown above the data where boxes represent exons (black boxes = translated regions, white

boxes = untranslated regions); the zigzag line represents an intron; and the straight line represents upstream sequence. Regions amplified by Egr-1 primers are represented by black bars.



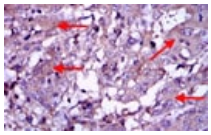
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT2 antibody [4H7] (ab175354)

Immunohistochemical analysis of deparaffinized Human Esophageal cancer tissue labeling AKT2 with ab175354 at 1/200 dilution. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate.



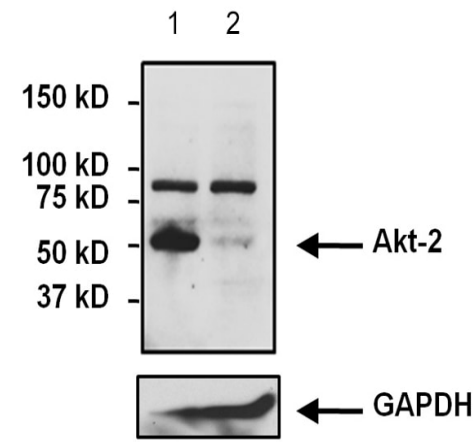
Immunoprecipitation - Anti-AKT2 antibody [4H7] (ab175354)

Immunoprecipitation of AKT2 was performed on HeLa cells. The antigen:antibody complex was formed by incubating 750 µg whole cell lysate with 2 µg of ab175354. WB detection used ab175354 at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT2 antibody [4H7] (ab175354)

Immunohistochemical analysis of deparaffinized normal Human Medulla Oblongata tissue labeling AKT2 with ab175354 at 1/200 dilution. Detection was performed using a goat anti-mouse HRP secondary antibody followed by colorimetric detection using DAB substrate.



Western blot - Anti-AKT2 antibody [4H7] (ab175354)

All lanes : Anti-AKT2 antibody [4H7] (ab175354) at 1/1000 dilution

Lane 1 : Non-transfected U2OS cells

Lane 2 : U2OS cells transfected with AKT2 siRNA

Secondary

All lanes : goat anti-mouse-HRP at 1/20000 dilution

Predicted band size: 55 kDa

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