abcam

Product datasheet

Anti-PAK2 antibody [EP796Y] - BSA and Azide free ab227990

KO VALIDATED Recombinant RabMAb

3 References 12 Images

Overview

Product name	Anti-PAK2 antibody [EP796Y] - BSA and Azide free
Description	Rabbit monoclonal [EP796Y] to PAK2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, NIH/3T3, RAW 264.7, Wild-type HEK-293T, PAK2 CRISPR-Cas9 edited HEK-293T and C6 cell lysates. IHC-P; Human breast carcinoma tissue IF/ICC: T47D cell line. Flow Cyt (intra): HeLa cells. IP: HeLa and NIH/3T3 cell lysates.
General notes	ab227990 is the carrier-free version of **ab76293**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20

	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP796Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227990 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
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 58 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. This antibody may not be suitable for IHC with mouse or rat samples Use of HRP conjugated or polymerized HRP secondary antibody is recommended. Stronger signals have been found using the
ICC/IF		Use at an assay dependent concentration.

Target

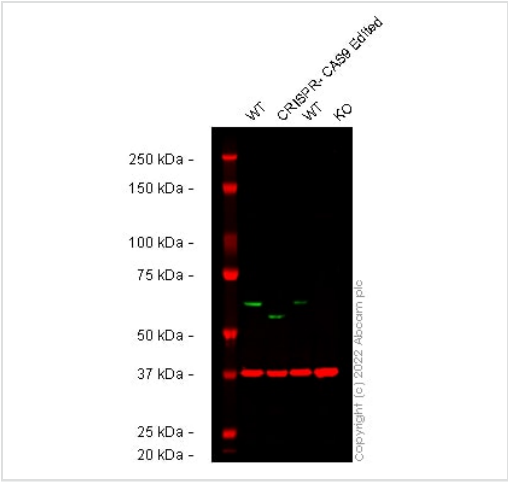
Function	The activated kinase acts on a variety of targets. Phosphorylates ribosomal protein S6, histone H4 and myelin basic protein. Full length PAK 2 stimulates cell survival and cell growth. The process is, at least in part, mediated by phosphorylation and inhibition of pro-apoptotic BAD. Caspase-activated PAK-2p34 is involved in cell death response, probably involving the JNK signaling pathway. Cleaved PAK-2p34 seems to have a higher activity than the CDC42-activated form.
Tissue specificity	Ubiquitously expressed. Higher levels seen in skeletal muscle, ovary, thymus and spleen.
Sequence similarities	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. STE20 subfamily. Contains 1 CRIB domain. Contains 1 protein kinase domain.
Post-translational modifications	Full length PAK 2 is autophosphorylated when activated by CDC42/p21. Following cleavage, both peptides, PAK-2p27 and PAK-2p34, become highly autophosphorylated, with PAK-2p27 being phosphorylated on serine and PAK-2p34 on threonine residues, respectively.

Autophosphorylation of PAK-2p27 can occur in the absence of any effectors and is dependent on phosphorylation of Thr-402, because PAK-2p27 is acting as an exogenous substrate.
During apoptosis proteolytically cleaved by caspase-3 or caspase-3-like proteases to yield active PAK-2p34.
Ubiquitinated, leading to its proteasomal degradation.
PAK-2p34 is myristoylated.

Cellular localization

Cytoplasm and Nucleus. Cytoplasm > perinuclear region. Membrane. Interaction with ARHGAP10 probably changes PAK-2p34 location to cytoplasmic perinuclear region. Myristoylation changes PAK-2p34 location to the membrane.

Images



Western blot - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

All lanes : Anti-PAK2 antibody [EP796Y] (**ab76293**) at 1/5000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PAK2 CRISPR-Cas9 edited HEK-293T cell lysate
Lane 3 : Wild-type HeLa **ab255552** cell lysate
Lane 4 : PAK2 knockout HeLa **ab260287** cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

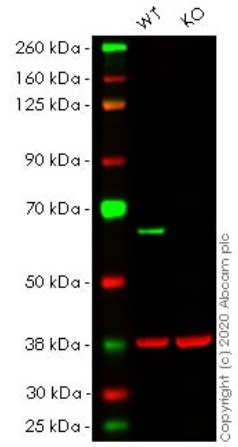
Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 65 kDa

False colour image of Western blot: Anti-PAK2 antibody [EP796Y] staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab76293** was shown to bind specifically to PAK2. A band was observed at 65 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in PAK2 CRISPR-Cas9 edited cell line **ab282648** (CRISPR-Cas9 edited cell lysate **ab283047**). The band observed in the CRISPR-Cas9 edited lysate lane below 65 kDa is likely to represent a truncated form of PAK2. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and PAK2 CRISPR-Cas9 edited

HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation (**ab76293**).



Western blot - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

All lanes : Anti-PAK2 antibody [EP796Y] (**ab76293**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PAK2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

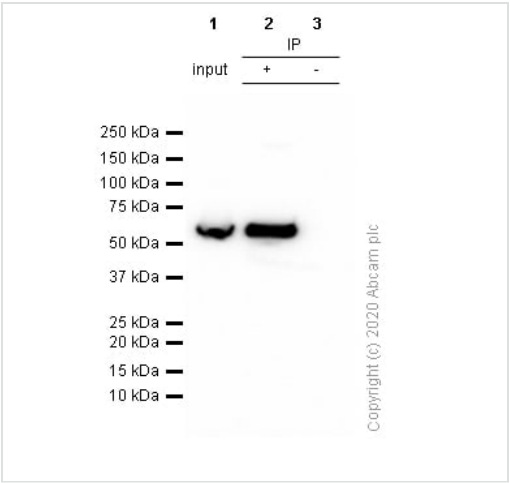
Predicted band size: 58 kDa
Observed band size: 60 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab76293**).

Lanes 1- 2: Merged signal (red and green). Green - **ab76293** observed at 60 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab76293 was shown to react with PAK2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264814** (knockout cell lysate **ab257573**) was used. Wild-type HeLa and PAK2 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab76293** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**)

secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

PAK2 was immunoprecipitated from 0.35 mg NIH/3T3 (Mouse embryonic fibroblast) cell lysate 10 µg with **ab76293** at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab76293** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (Mouse embryonic fibroblast) cell lysate 10 µg

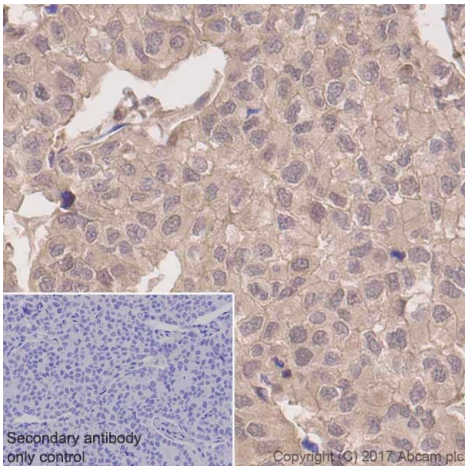
Lane 2: **ab76293** IP in NIH/3T3 cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab76293** in HeLa cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 7 seconds

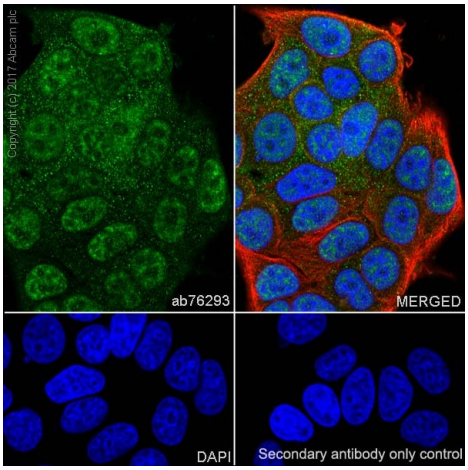
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling PAK2 with Purified **ab76293** at 1:100 dilution (2.02 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

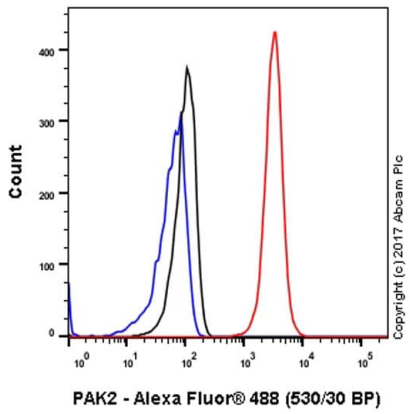
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).



Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling PAK2 with purified **ab76293** at 1:100 dilution (2.0µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

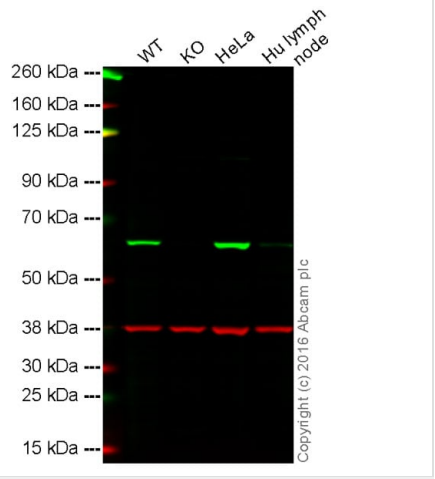
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).



Flow Cytometry (Intracellular) - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PAK2 with purified **ab76293** at 1/20 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).

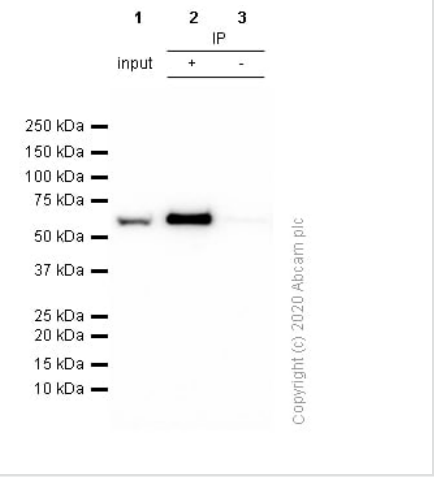


Western blot - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

This WB data was generated using the same anti-PAK2 antibody clone, EP796Y, in a different buffer formulation (cat# **ab76293**).

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PAK2 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human lymph node tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - **ab76293** observed at 60 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Unpurified **ab76293** was shown to specifically react with PAK2 when PAK2 knockout samples were used. Wild-type and PAK2 knockout samples were subjected to SDS-PAGE. **ab76293** and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunoprecipitation - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

PAK2 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate 10 µg with **ab76293** at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab76293** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate 10 µg

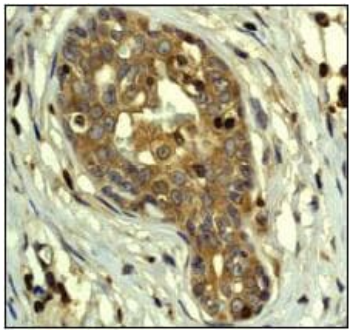
Lane 2: **ab76293** IP in HeLa cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab76293** in HeLa cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 7 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).

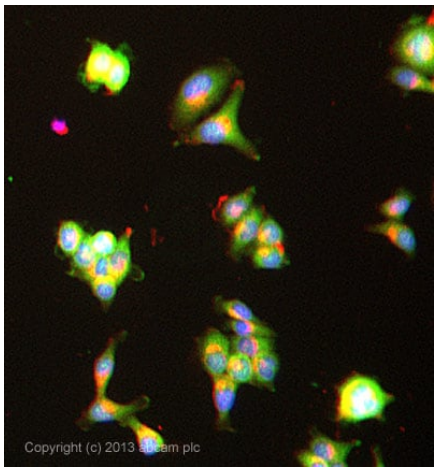


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

This IHC data was generated using the same anti-PAK2 antibody clone, EP796Y, in a different buffer formulation (cat# **ab76293**).

Unpurified **ab76293**, at a 1/100 dilution, staining PAK2 in paraffin embedded human breast carcinoma tissue by Immunohistochemistry.

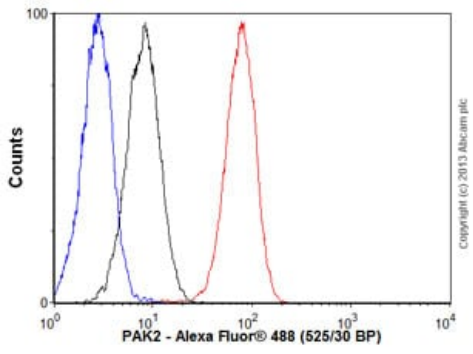
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

ICC/IF image of unpurified **ab76293** stained T47D cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab76293**, 1µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).

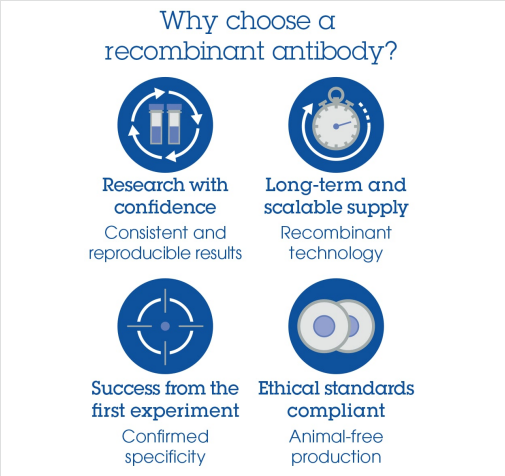


Flow Cytometry (Intracellular) - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

Overlay histogram showing HeLa cells stained with unpurified **ab76293** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab76293**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76293**).



Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

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