# abcam

## Product datasheet

# Anti-ATP6V1B2 antibody ab73404

# ★★★★★ 1 Abreviews 20 References 5 Images

#### Overview

Product name Anti-ATP6V1B2 antibody

**Description** Rabbit polyclonal to ATP6V1B2

Host species Rabbit

Tested applications Suitable for: IP, IHC-P, WB, ICC/IF

**Species reactivity** Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Cynomolgus monkey, Orangutan

**Immunogen** Synthetic peptide conjugated to KLH derived from within residues 450 to the C-terminus of Mouse

ATP6V1B2.Read Abcam's proprietary immunogen policy(Peptide available as ab74898.)

**Positive control**This antibody gave a positive signal in the following tissue lysates: Rat hippocampus; Mouse

hippocampus; Mouse kidney; Mouse testis; Human kidney; Human testis.

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 or -

 $80^{\circ}\text{C}\,.$  Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

**Purity** Immunogen affinity purified

1

**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab73404 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 a concentration of 5 µg/ml.
IHC-P	****(1)	Use a concentration of 0.1 - 0.5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 57 kDa (predicted molecular weight: 57 kDa).
ICC/IF		Use a concentration of 5 µg/ml.

#### **Target**

**Function** Non-catalytic subunit of the peripheral V1 complex of vacuolar ATPase. V-ATPase is responsible

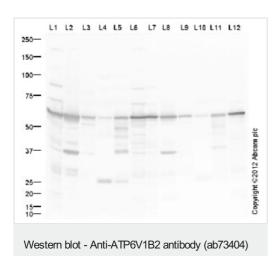
for acidifying a variety of intracellular compartments in eukaryotic cells.

**Sequence similarities**Belongs to the ATPase alpha/beta chains family.

**Cellular localization** Endomembrane system. Melanosome. Endomembrane. Identified by mass spectrometry in

melanosome fractions from stage I to stage IV.

#### **Images**



All lanes: Anti-ATP6V1B2 antibody (ab73404) at 1 µg/ml

Lane 1 : Hippocampus (Mouse) Tissue Lysate
Lanes 2 & 8 : Kidney (Mouse) Tissue Lysate

Lanes 3 & 9: Testis (Mouse) Tissue Lysate

Lanes 4 & 10: Human testis tissue lysate - total protein (ab30257)

Lanes 5 & 11: Human kidney tissue lysate - total protein

(ab30203)

Lanes 6 & 12 : Rat Hippocampus Tissue Lysate

Lane 7: Hippocampus (Mouse) Tissue Lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed

(HRP) (ab65484) at 1/3000 dilution

Performed under reducing conditions.

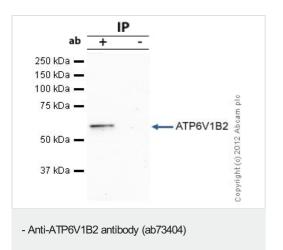
**Predicted band size:** 57 kDa **Observed band size:** 57 kDa

Additional bands at: 37 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 30 seconds

Lanes 1-6 were blocked with 5% BSA, Lanes 7-12 were blocked with 3% milk. Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.



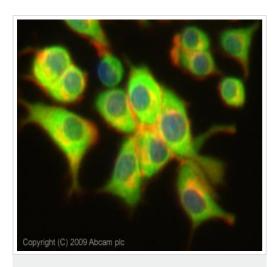
ATP6V1B2 was immunoprecipitated using 0.5mg Mouse Testis tissue lysate, 5µg of Rabbit polyclonal to ATP6V1B2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Mouse Testis tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab73404.

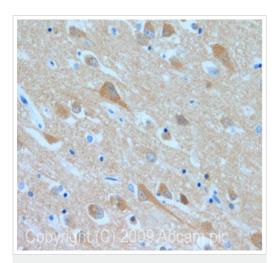
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

Band: 57kDa; ATP6V1B2



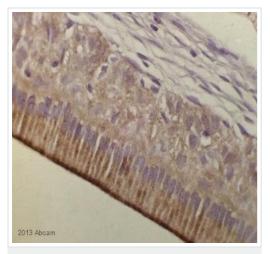
Immunocytochemistry/ Immunofluorescence - Anti-ATP6V1B2 antibody (ab73404)

ICC/IF image of ab73404 stained PC12 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 (ab73404, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit lgG (H+L) used at a 1/1000 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 antibody also gave a positive result in 4% PFA fixed (10 min) PC12 cells at 5µg/ml.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATP6V1B2 antibody (ab73404)

IHC image of ATP6V1B2 staining in human hippocampus formalin fixed paraffin embedded tissue section, performed on a Leica Bond  $^{TM}$  system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab73404, 0.5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATP6V1B2 antibody (ab73404)

This image is courtesy of an anonymous Abreview

ab73404 staining RAt incisor tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with 2.5% serum for 30 minutes at 22°C. Samples were incubated with primary antibody (1/1000 in 1% BSA/ 0.5% Triton X-100 in PBS) for 16 hours at 4°C. An undiluted peroxidase-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.

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