

Basic Information

Product Name	Anti-AMPK Beta 2/PRKAB2 Antibody	
Gene Name	PRKAB2	
Source	Rabbit	
Isotype	IgG	
Species Reactivity	human, mouse, rat	
Tested Application	WB, IHC, IHC-F, ICC/IF, FCM	
Contents	500 ug/ml antibody with PBS , 0.02% NaN ₃ , 1 mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human AMPK beta 2 (56-89aa DKEFVSWQQDLEDSVKPTQQARPTVIRWSEGGKE), different from the related mouse sequence by three amino acids, and from the related rat sequence by two amino acids.	
concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	34KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry in paraffin section (IHC): 1:50-400 Immunohistochemistry in frozen section (IHC-F): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow cytometry (FCM): 1-3 µg/1x10 ⁶ cells (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

5'-AMP-activated protein kinase subunit beta-2 is an enzyme that in humans is encoded by the PRKAB2 gene. The protein encoded by this gene is a regulatory subunit of the AMP-activated protein kinase (AMPK). AMPK is a heterotrimer consisting of an alpha catalytic subunit, and non-catalytic beta and gamma subunits. It is an important energy-sensing enzyme that monitors cellular energy status. In response to cellular metabolic stresses, AMPK is activated, and thus phosphorylates and inactivates acetyl-CoA carboxylase (ACC) and beta-hydroxy beta-methylglutaryl-CoA reductase (HMGCR), key enzymes involved in regulating de novo biosynthesis of fatty acid and cholesterol. This subunit may be a positive regulator of AMPK activity. It is highly expressed in skeletal muscle and thus may have tissue-specific roles. Multiple alternatively spliced transcript variants have been found for this gene.

Selected Validation Data

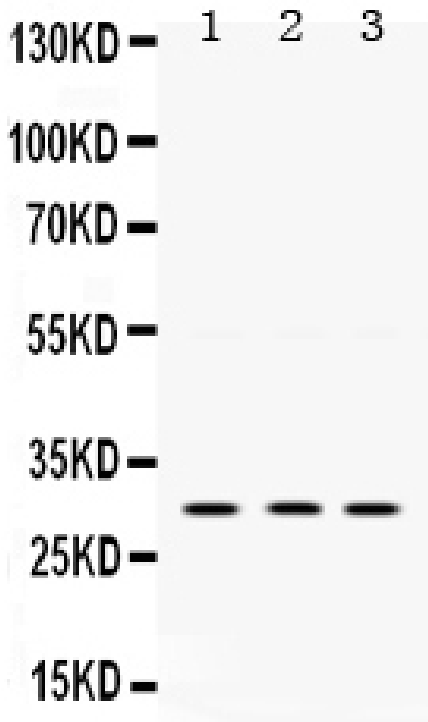


Figure 1. Western blot analysis of AMPK beta 2 using anti-AMPK beta 2 antibody (PB0723). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: Rat Brain Tissue Lysate, Lane 2: Rat Skeletal Muscle Tissue Lysate, Lane 3: PANC Whole Cell Lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-AMPK beta 2 antigen affinity purified polyclonal antibody (Catalog # PB0723) at 0.5 $\mu\text{g}/\text{mL}$ overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for AMPK beta 2 at approximately 30KD. The expected band size for AMPK beta 2 is at 30KD.

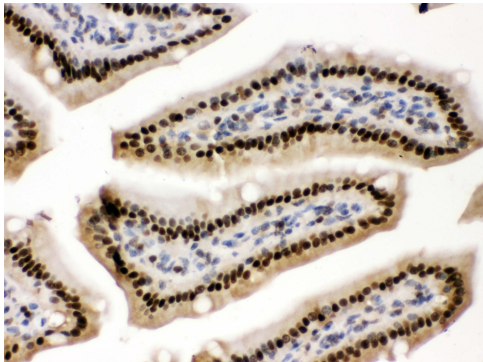


Figure 2. IHC analysis of AMPK beta 2 using anti-AMPK beta 2 antibody (PB0723). AMPK beta 2 was detected in paraffin-embedded section of Mouse Intestine Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu\text{g}/\text{ml}$ rabbit anti-AMPK beta 2 Antibody (PB0723) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

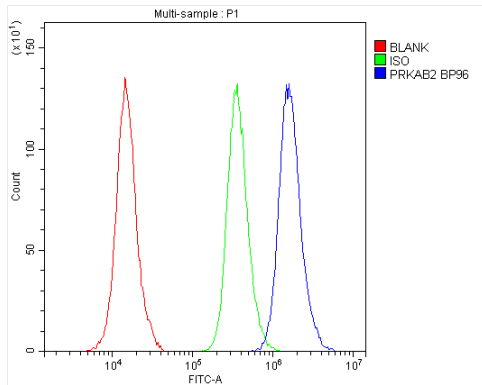


Figure 5. Flow Cytometry analysis of A431 cells using anti- AMPK beta 2 antibody (PB0723).

Overlay histogram showing A431 cells stained with PB0723 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti- AMPK beta 2 Antibody (PB0723,1μg/1x10⁶ cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 5-10μg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1μg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

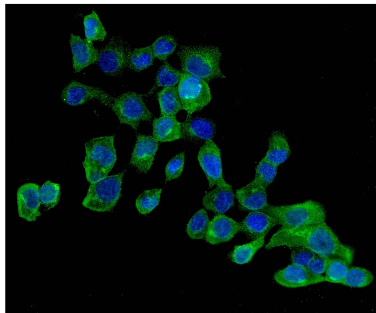


Figure 6. ICC analysis using anti-PRKAB2 antibody (PB0723) was detected in immersion fixed A431 cell line . Cells were stained using the Dylight488-conjugated Anti-rabbit IgG Secondary Antibody (green)(Catalog#?BA1127) and counterstained with DAPI (blue).