Datasheet for ABIN456151

# PRKAB1 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | PRKAB1 |
| Binding Specificity: | phosphospecific |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Detection Range: | 0.312-20 ng/mL |
| Minimum Detection Limit: | 0.312 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | This immunoassay kit allows for the use in vitro quantitative determination of human Phosphorylated adenosine monophosphate activated protein kinase, AMPK concentrations in tissue homogenates and other biological fluids. |
| Sample Type: | Tissue Homogenate |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This assay recognizes recombinant and natural human AMPK. |
| Cross-Reactivity (Details): | No significant cross-reactivity or interference was observed. |
| Sensitivity: | < 1 U/mL<br>The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest |

## Product Details

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| | detectable concentration that could be differentiated from zero. |
| Characteristics: | Homo sapiens,Human,5'-AMP-activated protein kinase subunit beta-1,AMPK subunit beta-1,AMPKb,PRKAB1,AMPK |
| Components: | Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay DiluentB 1 x 10ml Detection Reagent A (1x120µl), Detection Reagent B (1x120µl), Wash Buffer(25 x concentrate) (1x30ml), Substrate (1x10ml), Stop Solution (1x10ml) |

## Target Details

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| Target: | PRKAB1 |
| Alternative Name: | PRKAB1 (PRKAB1 Products) |
| Background: | 5'AMP-activated protein kinase or AMPK consists of three proteins (subunits) that together make a functional enzyme, conserved from yeast to humans, that plays a role in cellular energy homeostasis. It is expressed in a number of tissues, including the liver, brain, and skeletal muscle. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells. AMPK acts as a metabolic master switch regulating several intracellular systems including the cellular uptake of glucose, the beta-oxidation of fatty acids and the biogenesis of glucose transporter 4 (GLUT4) and mitochondria. The energy-sensing capability of AMPK can be attributed to its ability to detect and react to fluctuations in the AMP:ATP ratio that take place during rest and exercise (muscle stimulation). During muscle stimulation, AMP increases while ATP decreases, which changes AMPK into a good substrate for activation via an upstream kinase complex, AMPKK. AMPKK is a complex of three proteins, STE-related adaptor (STRAD), mouse protein 25 (MO25), and LKB1 (a serine/threonine kinase). During a bout of exercise, AMPK activity increases while the muscle cell experiences metabolic stress brought about by an extreme cellular demand for ATP. Upon activation, AMPK increases celluar energy levels by inhibiting anabolic energy consuming pathways (fatty acid synthesis, protein synthesis, etc.) and stimulating energy producing, catabolic pathways (fatty acid oxidation, glucose transport, etc.). Recent research on mice at Harvard University has shown that when the activity of AMPK was inhibited, the mice ate less and lost weight, but these data are controversial. When AMPK levels were artificially raised the mice ate more and gained weight. Research in Britain has shown that the appetite-stimulating hormone ghrelin also affects AMPK levels. A 2001 study (Zhou G et al) has indicated that the antidiabetic drug metformin (Glucophage®) acts by stimulating AMPK, |

## Target Details

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| | leading to reduced insulin resistance in the liver. Metformin usually 2 causes weight loss and reduced appetite, not weight gain and increased appetite, which is opposite of what might be expected given the Harvard mouse study results. |
| Pathways: | AMPK Signaling, Warburg Effect |

## Application Details

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| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Protocol: | The microtiter plate provided in this kit has been pre-coated with an antibody specific to AMPK. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for AMPK and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain AMPK, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of AMPK in the samples is then determined by comparing the O.D. of the samples to the standard curve. |
| Reagent Preparation: | Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 200 U/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (200 U/mL). The Sample Diluent serves as the zero standard (0 U/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively. |
| Sample Collection: | Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20 °C. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay |

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| | immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8C, otherwise samples must stored at -20 °C (≤ 3 months) or -80 °C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. It is recommended that all samples be assayed in duplicate. 3 |
| Assay Procedure: | Allow all reagents to reach room temperature. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.<br>1. Add 100 uL of Standard, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C.<br>2. Remove the liquid of each well, don't wash.<br>3. Add 100 uL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.<br>4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.<br>5. Add 100 uL of Detection Reagent B working solution to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37 °C.<br>6. Repeat the aspiration/wash as in step<br>4. 4<br>7. Add 90 uL of Substrate Solution to each well. Incubate within 30 minutes at 37°C. Protect from light.<br>8. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.<br>9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.<br>Important Note:<br>1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once.<br>2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. |

## Application Details

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| | 3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.<br>4. Duplication of all standards and specimens, although not required, is recommended.<br>5. When mixing or reconstituting protein solutions, always avoid foaming.<br>6. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.<br>7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.<br>8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. |
| Calculation of Results: | Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data 5 may be linearized by plotting the log of the AMPK concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | 1. The kit should not be used beyond the expiration date on the kit label.<br>2. Do not mix or substitute reagents with those from other lots or sources.<br>3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding.<br>4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded. |
| Storage: | 4 °C/-20 °C |

## Handling

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| Storage Comment: | The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C. |