CREATIVE BIOMART®
Assay Kit

AMPK Phosphorylation Assay Kit

Product Information

Cat.No. Kit-0088

Product Overview

AMPK Phosphorylation Assay Kit is an indirect enzyme-linked immunoassay for qualitative determination of AMPK phosphorylation in cultured cells. at FL360/450, 530/585 nm.

Description

The 5-AMP-activated protein kinase (AMPK) is a key sensor of intracellular energy balance. AMPK is activated in response to an increase in the AMP/ATP ratio which can be caused by a number of factors such as muscle contraction, starvation, or hypoxia. AMPK is a heterotrimeric protein complex comprising of a- (63 kDa), β- (38 kDa) and γ- (38 kDa) subunits. For each subunit, isoforms have been identified (a1, a2, β1, β2, γ1, γ2, γ3) which theoretically allow the formation of 12 different proteins. The a- subunit contains a serine/threonine kinase domain and the regulatory subunits contain binding sites for AMP and ATP (γ-subunit) and for glycogen (β-subunit). AMPK is activated by phosphorylation on Thr-172 within the catalytic domain. AMP binding results in a 2 to 5-fold increase in AMPK activity compared to the basal level. Binding of AMP to the γ-subunit causes allosteric activation of the kinase and induces a conformational change in the kinase domain that protects AMPK from dephosphorylation of Thr-172. AMPK Phosphorylation Assay Kit measures phosphorylated AMPK in whole cells and normalizes the signal to the total protein content. The antibody recognizes both a-subunits and thus can be used for cells from all tissues (human, mouse, rat). This simple and efficient assay eliminates the need for cell lysate preparation and can be used to study AMPK regulation in short-term and long-term assays. In this assay, cells grown in 96-well plates are fixed and permeabilized in the wells. AMPK phosphorylation (pAMPK) is measured using a fluorescent ELISA followed by total protein measurement in each well.

Applications

Determination of AMPK phosphorylation status in whole cells.Evaluation of direct and indirect modulation of AMPK phosphorylation.Species tested: human, mouse, rat.

Target Species

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AMPK Phosphorylation Assay Kit

Human, Mouse, Rat

Usage

For research use only (RUO)

Storage

Upon delivery, store all reagents at -20°C. Shelf life: 6 months after receipt.

Kit Components

10x Wash Buffer 25 mLProtein Stain 6 mLAb1 10 μLBlocking Buffer 25 mLHRP Substrate 6 mLAb2 10 μL

Detection method Fluorimetric

Compatible Sample Types

Cell

Features & Benefits

Sensitive. Can measure pAMPK modulation in as little as 500 cells/well.Simple and convenient. No cell lysis necessary, cells can be cultured for several days.

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