CREATIVE BIOMART®
Assay Kit

PDK1 Kinase (Human) Assay/Inhibitor Screening Assay Kit

Product Information

Cat.No. Kit-0672

Product Overview

PDK1 Kinase (Human) Assay/Inhibitor Screening Assay Kit is a single-site, non-quantitative immunoassay for PDK1 activity. Plates are pre-coated with a substrate-peptide corresponding to surrounding AKT1 T308, which contains threonine residues that can be efficiently phosphorylated by PDK1. The detector antibody specifically detects only the phosphorylated form of threonine residue on substrate-peptide.

Description

PDK1 (3-phosphoinositide-dependent protein kinase-1) plays an importantrole in insulin and growth factor signalling cascades. It is known to phosphorylate and activate at least 24 protein kinases that belong to the AGC (cAMP-dependent, cGMP-dependent, protein kinase C) family of protein kinases. These comprise isoforms of Akt /PKB, also, p70 ribosomal S6-kinase, serum and glucocorticoid responsive kinase, p90 ribosomal S6 kinase and PKC. PDK1 activates these enzymes by phosphorylating a Ser/Thr residue in their activation loop. PDK1 is a 556 amino-acid protein comprising an N-terminal Ser/Thr kinase catalytic domain (residues 71−359) and a C-terminal pleckstrin homology (PH) domain (residues 459−550). The latter binds with high affinity to the second messenger phosphatidylinositol-3,4,5-trisphosphate, generated through insulin- and growth factor-induced activation of PI3-kinase.

Applications

1) Screening inhibitors or activators of PDK1.2) Detecting the effects of pharmacological agents on PDK1 activity.

Target Species

Human

Usage

For research use only (RUO)

Storage

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Upon receipt store the ATP at -20°CUpon receipt, store all other components at 4°C. Do not expose reagents to excessive light

Kit Components

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with AKT308-peptide as PDK1 substrate.10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween -20.Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.20X ATP: Lyophilized ATP Na2 salt. Reconstitute contents of vial with 1.6 mL of H2O. Mix gently until dissolved. Final concentration of ATP should be 1.25 mM ATP. The ATP solution can be stored in small aliquots (e.g. 100 µL) at -20°C. The 1.25 mM ATP stock solution must be diluted to 62.5 µM in Kinase Reaction Buffer at the time of the assay.Anti-Phospho-AKT T308 Polyclonal Antibody (PPT-08): One vial containing 12 mL of anti-phospho-AKT T308 polyclonal antibody (PPT-08).Ready to use.HRP conjugated Anti-rabbit IgG: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-rabbit IgG. Ready to use.Substrate Reagent: 12 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.Stop Solution: One bottle supplied ready to use, containing 12 mL of 0.5 N H2SO4.

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