

AKT/PKB Kinase (Human) Assay/Inhibitor Screening Kit

Cat. No. Kit-0066 **Lot. No.** (See product label)

SPECIFICATION

Product Overview

AKT/PKB Kinase (Human) Assay/Inhibitor Screening Kit is a single-site, non-quantitative immunoassay for AKT activity. Plates are pre-coated with "AKTide-2T" (10), which can be efficiently phosphorylated by AKT1, 2 and 3 on a microtiter plate. The detector antibody is AT-3E2, an antibody that specifically detects only the phosphorylated "AKTide-2T".

Description

The AKT oncogene was isolated from the directly transforming murine retrovirus AKT8, which was isolated from an AKR mouse thymoma cell line. Staal cloned the human cellular homolog, AKT1. He found a 20-fold amplification of the AKT1 gene in 1 of 5 gastric adenocarcinomas tested. Phosphoinositide 3-kinases (PI3Ks) generate specific inositol lipids that have been implicated in the regulation of cell growth, proliferation, survival, differentiation and cytoskeletal changes. One of the best characterized targets of PI3K lipid products is the protein kinase AKT or protein kinase B (PKB). In quiescent cells, AKT resides in the cytosol in a low-activity conformation. Upon cellular stimulation, AKT is activated through recruitment to cellular membranes by PI3K lipid products and phosphorylation by 3"-phosphoinositide-dependent kinase-1 (PDK1).

Applications

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

Target Species

Human

Usage

For research use only (RUO)

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Storage

Upon receipt store the ATP at -20°C Upon 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 AKTide-2T as an AKT 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 0.8 mL of H₂O. HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-phospho-AKTide-2T (AT-3E2) monoclonal antibody. Ready to use. Substrate Reagent: 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use. Stop Solution: One bottle supplied ready to use, containing 20 mL of 1 N H₂SO₄. Ready to use.

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