

ClariCELL™ PDK1/AKT1KD Kinase Assay Service

Description

The ClariCELL™ PDK1/AKT1KD Kinase Assay quantifies PDK1 dependent phosphorylation of a human full-length physiological substrate, kinase deficient AKT1 (AKT1KD), in human cells. The assay is useful to determine potencies of small-molecule inhibitors against the specified kinase in the context of a cellular environment. Compound testing services are available utilizing the assay.

Overview

Human Embryonic Kidney (HEK 293) cells transiently expressing sequence verified human full-length PDK1 and AKT1KD are exposed to test compound or control, then lysed to release cellular proteins. AKT1KD is captured onto an assay plate, and the extent of phosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells expressing substrate alone (AKT1KD) are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

PDK1 Expression in Cells

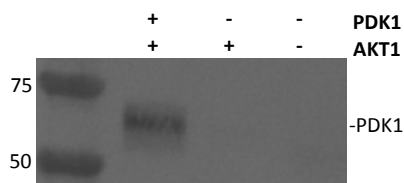


Figure 1: AKT1KD was expressed with or without wild type (Wt) PDK1 transiently in 293 cells. Following cell lysis, a Western was performed with appropriate antibodies to detect total PDK1 protein.

AKT1KD Phosphorylation in Cells

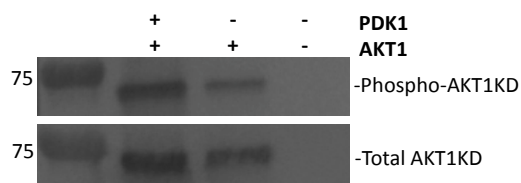


Figure 2: AKT1KD was expressed with or without wild type (Wt) PDK1 transiently in 293 cells. An IP Western was performed with appropriate antibodies to capture and detect phospho-AKT1KD and total AKT1 protein.

Quantification of Phosphorylation

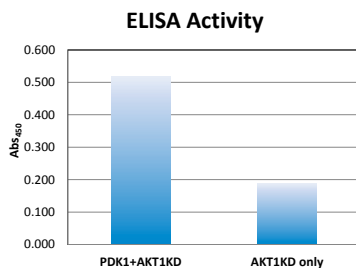


Figure 3: AKT1KD was expressed with or without wild type (Wt) PDK1 transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of phosphorylation of AKT1KD.

Reference Inhibitor Data

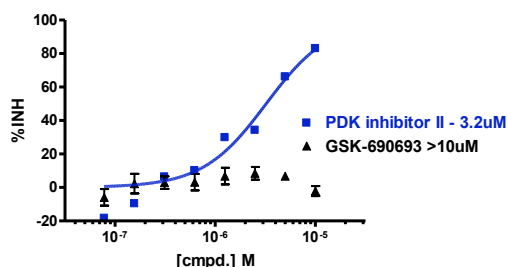


Figure 4: A phosphorylation assay was performed in the presence of PDK inhibitor II, and GSK-690693, a compound that is not expected to inhibit PDK1. % inhibition data were plotted to determine EC50s.