ClariCELL™ AKT1/BAD Kinase Assay Service

Description

The ClariCELL™ AKT1/BAD Kinase Assay quantifies AKT1 dependent phosphorylation of human full-length physiological substrate (BAD) 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 AKT1 and BAD are exposed to test compound or control, then lysed to release cellular proteins. BAD 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 kinase deficient AKT1 [K179M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

AKT1 Expression in Cells

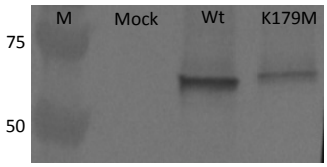

Figure 1: Wild type (Wt) or kinase dead (K179M) AKT1 was expressed with BAD transiently in 293 cells. Following cell lysis, a Western was performed with appropriate antibodies to detect total AKT1 protein.

BAD Phosphorylation in Cells

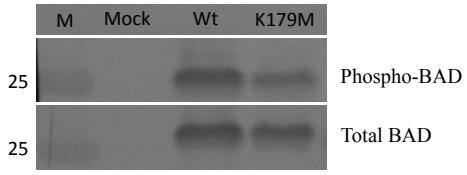

Figure 2: Wild type (Wt) or kinase dead (K179M) AKT1 was expressed with BAD transiently in 293 cells. An IP Western was performed with appropriate antibodies to capture and detect phospho-BAD and total BAD protein.

Quantification of Phosphorylation

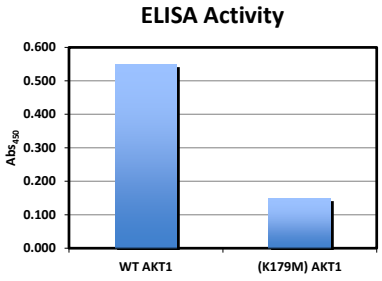

Figure 3: Wild type (WT) or kinase dead (K179M) AKT1 was expressed with BAD transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of phosphorylation of BAD.

Reference Inhibitor Data

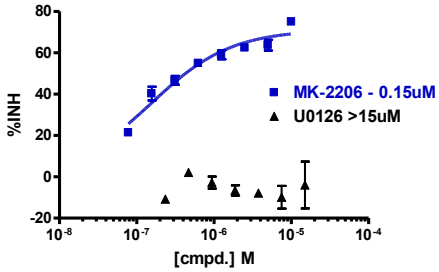

Figure 4: A phosphorylation assay was performed in the presence of MK-2206, an AKT1 inhibitor, and U0126, a compound that is not expected to inhibit AKT1. % inhibition data were plotted to determine EC50s.