ClariCELL® ITK Kinase Assay Service

Description

The ClariCELL ITK Kinase Assay quantifies autophosphorylation of human full-length ITK 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 ITK are exposed to test compound or control, then lysed to release cellular proteins. ITK is captured onto an assay plate, and the extent of autophosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells expressing kinase deficient ITK [K391M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

ITK Expression in Cells

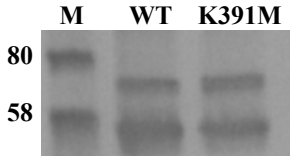

Figure 1: Wild type (wt) or kinase dead (K391M) ITK was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total ITK protein.

ITK Autophosphorylation in Cells

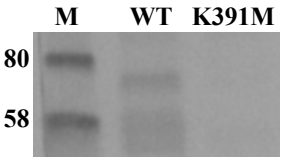

Figure 2: Wild type (wt) or kinase dead (K391M) ITK was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-ITK protein.

Quantification of Phosphorylation

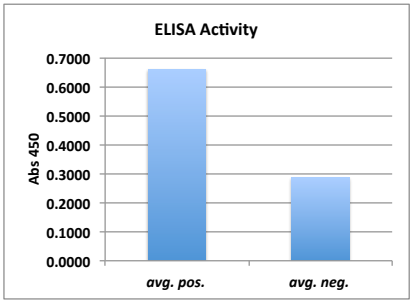

Figure 3: Wild type (wt) or kinase dead (K391M) ITK was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of ITK.

Reference Inhibitor Data

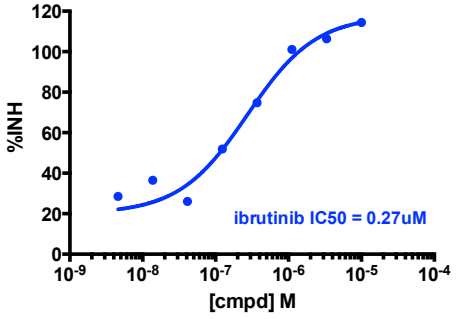

Figure 4: An autophosphorylation assay was performed in the presence of ibrutinib, an ITK inhibitor. Percent inhibition data were plotted to determine the IC50.