ClariCELL™ JAK3 Kinase Assay Service

Description

The ClariCELL™ JAK3 Kinase Assay quantifies autophosphorylation of human full-length JAK3 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 JAK3 are exposed to test compound or control, then lysed to release cellular proteins. JAK3 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 JAK3 [K855M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

JAK3 Expression in Cells

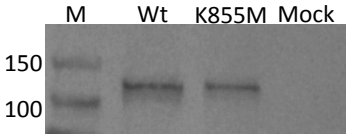

Figure 1: Wild type (wt) or kinase dead (K855M) JAK3 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total JAK3 protein.

JAK3 Autophosphorylation in Cells

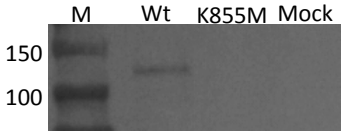

Figure 2: Wild type (wt) or kinase dead (K855M) JAK3 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-JAK3 protein.

Quantification of Phosphorylation

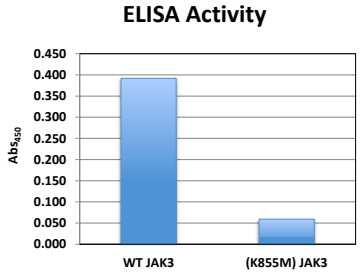

Figure 3: Wild type (wt) or kinase dead (K855M) JAK3 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of JAK3.

Reference Inhibitor Data

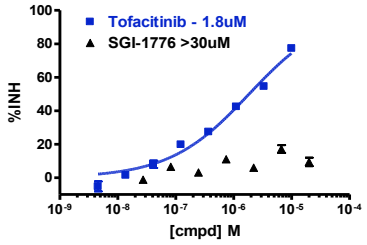

Figure 4: An autophosphorylation assay was performed in the presence of tofacitinib, a JAK3 inhibitor, and SGI-1776, a compound that is not expected to inhibit JAK3. % inhibition data were plotted to determine EC50s.

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