ClariCELL® JAK2 [V617F] Kinase Assay Service

Description

The ClariCELL® JAK2 [V617F] Kinase Assay quantifies autophosphorylation of human full-length constitutively active JAK2 [V617F] 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 JAK2 [V617F] are exposed to test compound or control, then lysed to release cellular proteins. JAK2 [V617F] 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 JAK2 [K882M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

JAK2 [V617F] Expression in Cells

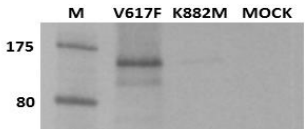

Figure 1: The constitutively active (V617F) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total mutant JAK2 protein.

JAK2 [V617F] Phosphorylation in Cells

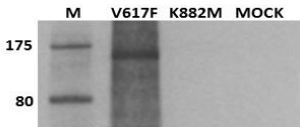

Figure 2: The constitutively active (V617F) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-JAK2 mutant protein.

Quantification of Phosphorylation

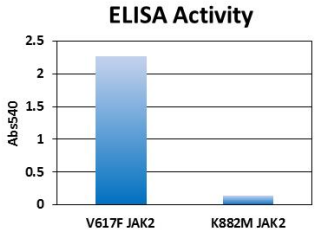

Figure 3: The constitutively active (V617F) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of JAK2 [V617F].

Reference Inhibitor Data

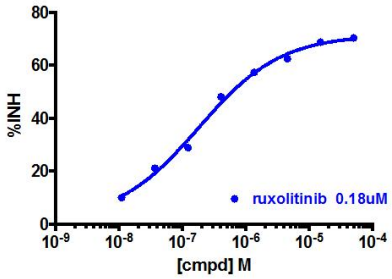

Figure 4: An autophosphorylation assay was performed in the presence of ruxolitinib, a JAK2 inhibitor that is also expected to inhibit JAK2 [V617F]. % inhibition data were plotted to determine the IC50.