

ClariCELL™ JAK2 Kinase Assay Service

Description

The ClariCELL™ JAK2 Kinase Assay quantifies autophosphorylation of human full-length JAK2 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 are exposed to test compound or control, then lysed to release cellular proteins. JAK2 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 validate that the assay is specific for kinase activity.

Assay Validation

JAK2 Expression in Cells

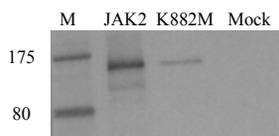


Figure 1: Wild type (wt) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total JAK2 protein.

JAK2 Autophosphorylation in Cells

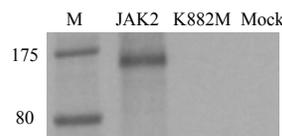


Figure 2: Wild type (wt) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-JAK2 protein.

Quantification of Phosphorylation

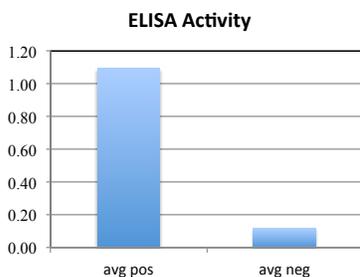


Figure 3: Wild type (wt) or kinase dead (K882M) JAK2 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of JAK2.

Reference Inhibitor Data

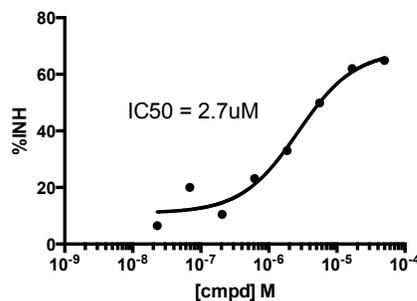


Figure 4: An autophosphorylation assay was performed in the presence of ruxolitinib, a JAK2 inhibitor. Percent inhibition data were plotted to determine the IC50.