

ClariCELL® JAK1 Kinase Assay Service

Description

The ClariCELL® JAK1 Kinase Assay quantifies autophosphorylation of human full-length JAK1 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 JAK1 are exposed to test compound or control, then lysed to release cellular proteins. JAK1 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 JAK1 [K908M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

JAK1 Expression in Cells

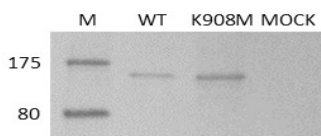


Figure 1: Wild type (WT) or kinase dead (K908M) JAK1 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total JAK1 protein.

JAK1 Phosphorylation in Cells

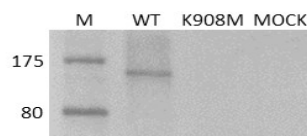


Figure 2: Wild type (WT) or kinase dead (K908M) JAK1 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-JAK1 protein.

Quantification of Phosphorylation

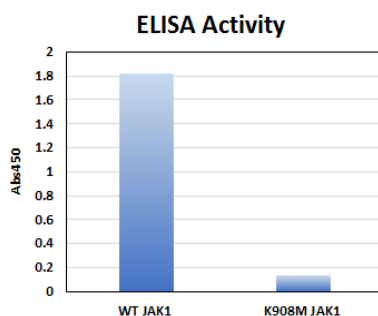


Figure 3: Wild type (WT) or kinase dead (K908M) JAK1 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of phosphorylation of JAK1.

Reference Inhibitor Data

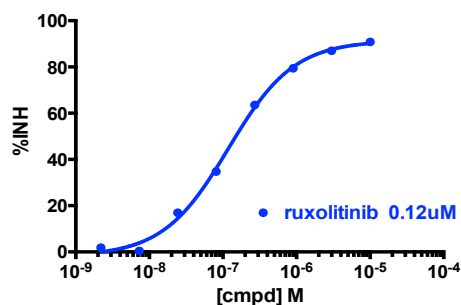


Figure 4: An ELISA phosphorylation assay was performed in the presence of ruxolitinib, a JAK1 inhibitor. Percent inhibition data were plotted to determine the IC₅₀.