

ClariCELL™ Tec Kinase Assay Service

Description

The ClariCELL™ Tec Kinase Assay quantifies autophosphorylation of human full-length Tec 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 Tec are exposed to test compound or control, then lysed to release cellular proteins. Tec 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 Tec [K398M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

Tec Expression in Cells

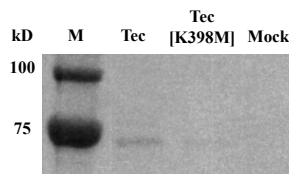


Figure 1: Wild type (wt) or kinase dead (K398M) Tec was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total Tec protein.

Tec Autophosphorylation in Cells

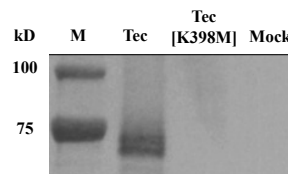


Figure 2: Wild type (wt) or kinase dead (K398M) Tec was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho- Tec protein.

Quantification of Phosphorylation

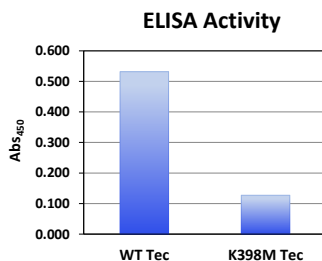


Figure 3: Wild type (wt) or kinase dead (K398M) Tec was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of Tec.

Reference Inhibitor Data

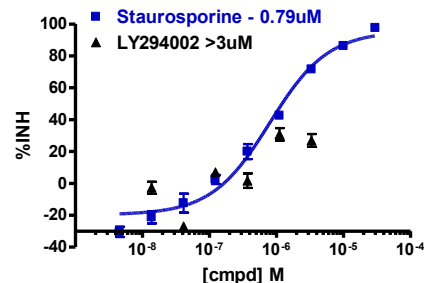


Figure 4: An autophosphorylation assay was performed in the presence of staurosporine, a non-selective Tec kinase inhibitor, and LY294002, a compound that does not inhibit Tec. % inhibition data were plotted to determine EC₅₀s.