

## ClariCELL™ MST1 (STK4) Kinase Assay Service

### Description

The ClariCELL™ MST1 Kinase Assay quantifies autophosphorylation of human full-length MST1 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 MST1 are exposed to test compound or control plus stimulus (okadaic acid), then lysed to release cellular proteins. MST1 is captured onto an assay plate, and the extent of autophosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells that are not stimulated with okadaic acid are utilized as controls to calculate the % inhibition of test compounds.

### Assay Validation

#### MST1 Expression in Cells

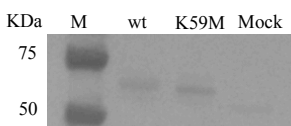


Figure 1: Wild type (wt) or kinase dead (K59M) MST1 was expressed transiently in 293 cells followed by stimulation with okadaic acid. After cell lysis, a Western was performed with appropriate antibodies to detect total MST1 protein.

#### MST1 Autophosphorylation in Cells

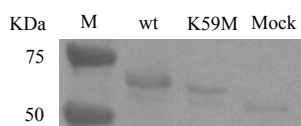


Figure 2: Wild type (wt) or kinase dead (K59M) MST1 was expressed transiently in 293 cells followed by stimulation with okadaic acid. After cell lysis, a Western was performed with appropriate antibodies to detect phospho-MST1 protein.

#### Quantification of Phosphorylation

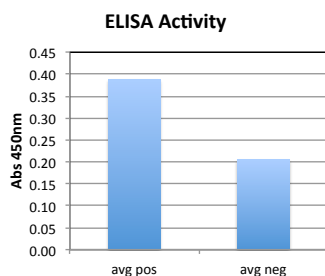


Figure 3: MST1 was expressed transiently in 293 cells either with (avg pos) or without (avg neg) okadaic acid treatment. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of MST1.

#### Reference Inhibitor Data

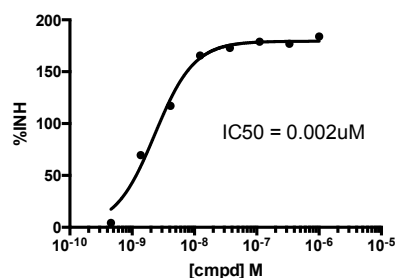


Figure 4: An autophosphorylation assay was performed in the presence of staurosporine, a MST1 inhibitor. % inhibition data were plotted to determine the IC50. A % inhibition of >100% is attributed to the non-specificity of staurosporine, as well as endogenous MST1 expression.