ClariCELL™ FGFR1 Kinase Assay Service

Description

The ClariCELL™ FGFR1 Kinase Assay quantifies autophosphorylation of human full-length FGFR1 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 FGFR1 are exposed to test compound or control, then lysed to release cellular proteins. FGFR1 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 FGFR1 [K512M] are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

FGFR1 Expression in Cells

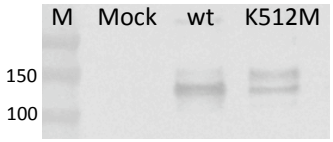

Figure 1: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total FGFR1 protein.

FGFR1 Autophosphorylation in Cells

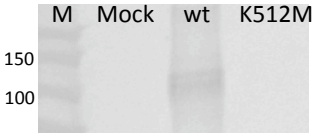

Figure 2: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho- FGFR1 protein.

Quantification of Phosphorylation

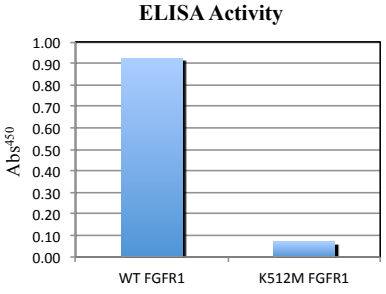

Figure 3: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of FGFR1.

Reference Inhibitor Data

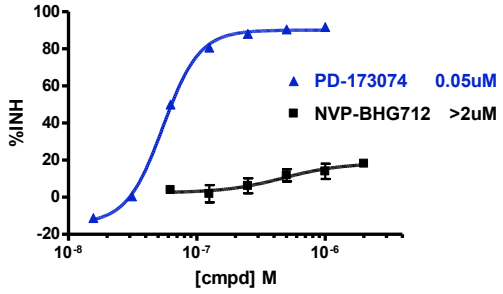

Figure 4: An autophosphorylation assay was performed in the presence of PD-173074, an FGFR1 inhibitor, and NVP-BHG712, a compound that is not expected to inhibit FGFR1. % inhibition data were plotted to determine EC50s.