## **Example of Assay Validation and Study**





## Description

The ClariCELL<sup>™</sup> FGFR1 Kinase Assav guantifies 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 guantified 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.

## To learn more, please contact

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# **ClariCELL™ FGFR1** Kinase Assay Service

**4-Step Assay Validation** 

### **FGFR1 Expression in Cells** Mock wt K512M M



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. 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 autophosphorvlation 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 EC50 values.

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