# ClariCELL™ EPHB4 Kinase Assay Service

## Description

The ClariCELL™ EPHB4 Kinase Assay quantifies autophosphorylation of human full-length EPHB4 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 EPHB4 are exposed to test compound or control, then lysed to release cellular proteins. EPHB4 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 EPHB4 [K647M] are also utilized as controls to calculate the % inhibition of test compounds.

## Assay Validation

### EPHB4 Expression in Cells

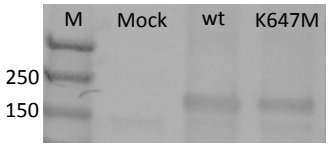


*Figure 1: Wild type (wt) or kinase dead (K647M) EPHB4 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total EPHB4 protein.*

### EPHB4 Autophosphorylation in Cells

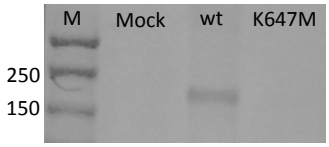


*Figure 2: Wild type (wt) or kinase dead (K647M) EPHB4 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-EPHB4 protein.*

### Quantification of Phosphorylation

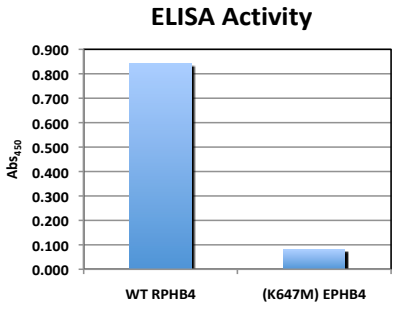


*Figure 3: Wild type (wt) or kinase dead (K647M) EPHB4 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of EPHB4.*

### Reference Inhibitor Data

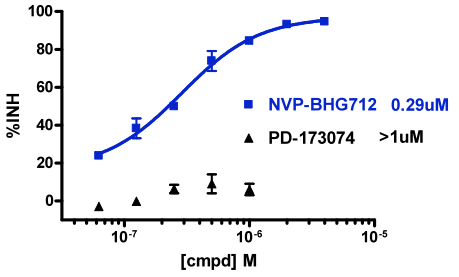


*Figure 4: An autophosphorylation assay was performed in the presence of NVP-BHG712, an EPHB4 inhibitor, and PD-173074, a compound that is not expected to inhibit EPHB4. % inhibition data were plotted to determine EC50s.*