

## ClariCELL™ BTK Kinase Assay Service

### Description

The ClariCELL™ BTK Kinase Assay quantifies autophosphorylation of human full-length BTK 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 BTK are exposed to test compound or control, then lysed to release cellular proteins. BTK 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 BTK [K430R] are also utilized as controls to calculate the % inhibition of test compounds.

### Assay Validation

#### **BTK Expression in Cells**

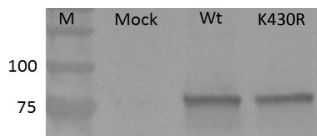


Figure 1: Wild type (wt) or kinase dead (K430R) BTK was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total BTK protein.

#### **BTK Autophosphorylation in Cells**

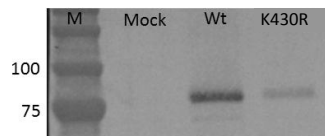


Figure 2: Wild type (wt) or kinase dead (K430R) BTK was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect phospho-BTK protein.

#### **Quantification of Phosphorylation**

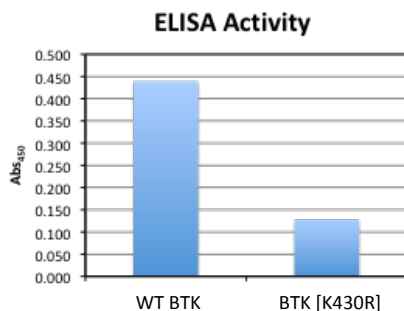


Figure 3: Wild type (WT) or kinase dead (K430R) BTK was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of BTK.

#### **Reference Inhibitor Data**

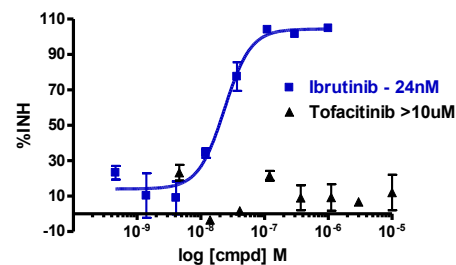


Figure 4: An autophosphorylation assay was performed in the presence of ibrutinib, a BTK inhibitor, and tofacitinib, a compound that is not expected to inhibit BTK. % inhibition data were plotted to determine EC<sub>50</sub>s.