



ClariCELL™ BTK [T474M] Kinase Assay Service

4-Step Assay Validation

BTK [T474M] Expression in Cells

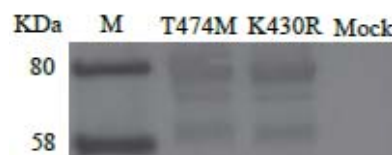


Figure 1: BTK [T474M] or kinase dead (K430R) BTK was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect the total mutant BTK protein.

BTK [T474M] Autophosphorylation in Cells



Figure 2: BTK [T474M] 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 [T474M] protein.

Quantification of Phosphorylation

ELISA Activity

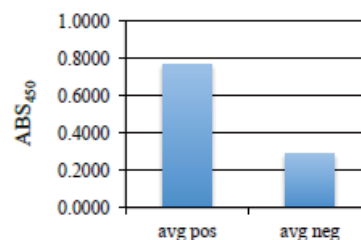


Figure 3: BTK [T474M] or kinase dead (K430R) BTK was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of auto-phosphorylation of BTK [T474M].

Reference Inhibitor Data

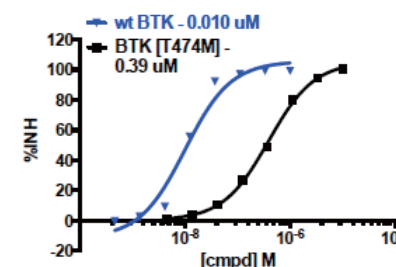


Figure 4: An autophosphorylation assay was performed for BTK and BTK [T474M] in the presence of ibrutinib, a BTK inhibitor. % inhibition data were plotted to determine EC₅₀s.

Description



The ClariCELL™ BTK [T474M] Kinase Assay quantifies autophosphorylation of human full-length BTK [T474M], a mutant form of BTK that is less sensitive to ibrutinib, 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 [T474M] are exposed to test compound or control, then lysed to release cellular proteins. BTK [T474M] 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.

サービスの詳細、最新情報はHPをご覧ください

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