ClariCELL™ DYRK1A/Tau Kinase Assay Service

Description

The ClariCELL™ DYRK1A/Tau Kinase Assay quantifies DYRK1A dependent phosphorylation of a human full-length physiological substrate, the microtubule-associated protein Tau, 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 DYRK1A and Tau are exposed to test compound or control, then lysed to release cellular proteins. Tau is captured onto an assay plate, and the extent of phosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells expressing substrate alone (Tau) are also utilized as controls to calculate the % inhibition of test compounds.

Assay Validation

DYRK1A Expression in Cells

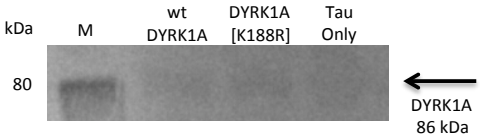

Figure 1: Human Tau was expressed with wild type (wt) DYRK1A, kinase deficient DYRK1A [K188R], or alone in 293 cells. Following cell lysis, a Western was performed with antibodies to detect total DYRK1A protein.

Tau Phosphorylation in Cells

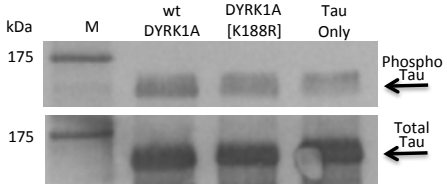

Figure 2: Human Tau was expressed with wild type (wt) DYRK1A, kinase deficient DYRK1A [K188R], or alone in 293 cells. An IP Western was performed with antibodies to capture and detect phospho-Tau and total Tau protein.

Quantification of Phosphorylation

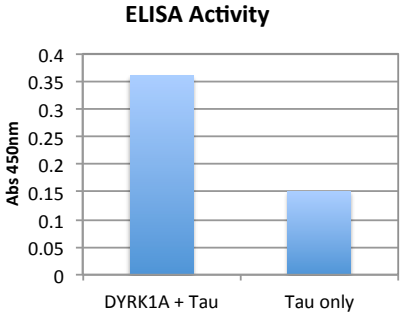

Figure 3: Tau was expressed with or without wild type DYRK1A in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of phosphorylation of Tau.

Reference Inhibitor Data

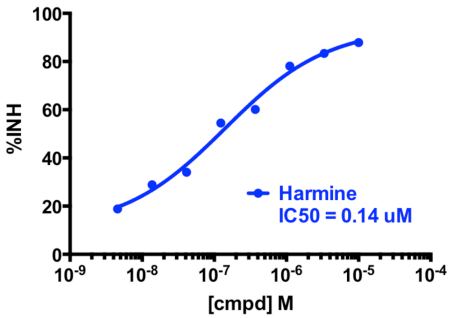

Figure 4: A phosphorylation assay was performed in the presence of harmine, a known DYRK1A inhibitor. Percent inhibition data were plotted to determine the IC50.