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Title: ERK Phosphorylation Status Screening Service

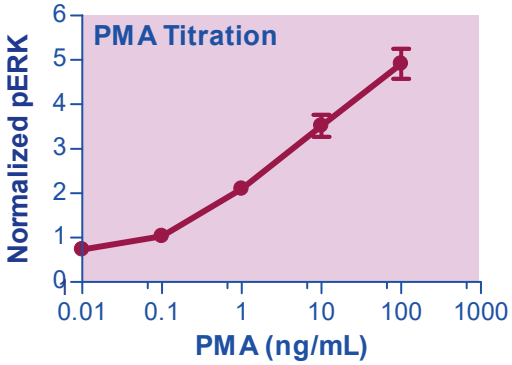
Summary

Using our EnzyFluo™ ERK Phosphorylation Assay Kit, we screen for modulators that increase or decrease ERK phosphorylation status in cells. We measure the ERK phosphorylation kinetics, EC50, and IC50 of cells treated with modulators. Testing can be run on a wide variety of cell lines.

Results

Phosphorylation Titration

To determine the time course of ERK phosphorylation, first a titration was run on the concentration of the test compound, here we use phorbol myristate acetate (PMA) as an example. In a sterile, black 96-well plate, PANC-1 cells were plated at a cell density of 10,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with medium dosed with PMA at the following concentrations: 10^{2}, 10^{1}, 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 0 µg/mL. The dosed culture medium was prepared from a stock solution of PMA in DMSO so that the final concentration of DMSO in the medium was 1%. Triplicate wells were incubated for 30 minutes with the PMA. The pERK and total protein was then quantified using the ERK Phosphorylation Assay Kit (EERK-100). The pERK values were normalized to the total protein content of the sample.



Phosphorylation Kinetics

After the titration on the test compound concentration, we measure the ERK phosphorylation kinetics for a specific dosage. We will continue to use PMA as an example here; we measure the ERK phosphorylation kinetics for PMA at a concentration of 100 ng/mL. In a sterile, black 96-well plate, PANC-1 cells were plated at a cell density of 10,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with 100 ng/mL PMA dosed medium. The dosed culture medium was prepared from a stock solution of PMA in DMSO so that the final concentration of DMSO in the medium was 1%. Triplicate wells were incubated for 2.5, 5, 10, 20, and

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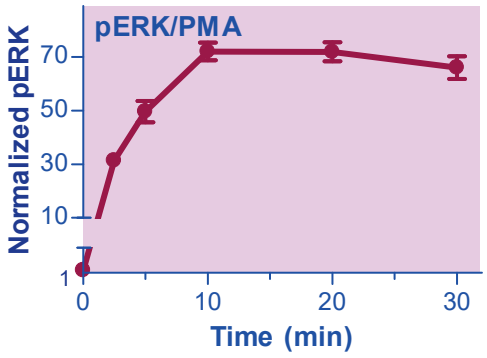
30 minutes with the PMA. The pERK and total protein was then quantified using the ERK Phosphorylation Assay Kit (EERK-100). The pERK values were normalized to the total protein content of the sample.



Inhibition Titration and IC50 Determination

To determine the IC50 of ERK phosphorylation inhibitors, we run a titration on the concentration of the test compound; here we use the drug Sorafenib as an example. The IC50 of Sorafenib, an ERK phosphorylation inhibitor, was determined for three cell lines: PANC-1, NIH-3T3, and RBL-2H3. The three cell lines were plated in sterile, black 96-well plates at a cell density of 10,000 cells per well and allowed to adhere overnight. The culture medium was aspirated and replaced with culture medium dosed with Sorafenib at the following concentrations: 10^{-2}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M. The dosed culture medium was prepared from a stock solution of Sorafenib in DMSO so that the final concentration of DMSO in the medium was 1%. Negative control wells received Sorafenib free culture medium with 1% DMSO. All treatment conditions were run in triplicate. The cells were incubated for three hours with the Sorafenib. After the three hour incubation, the Sorafenib medium was aspirated and replaced with 100 ng/mL PMA dosed medium. The cells were incubated with the PMA medium for five minutes. The pERK and total protein was then quantified using the ERK Phosphorylation Assay Kit (EERK-100). The pERK values were normalized to the total protein content of the sample. IC50 values were calculated using Graphpad Prism. Sorafenib IC50 values were calculated to be 2.1, 11.4 and 11.5 µM respectively, for RBL-2H3, NIH 3T3 and PANC-1 cell lines.