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Title: AMPK Phosphorylation Status Screening Service

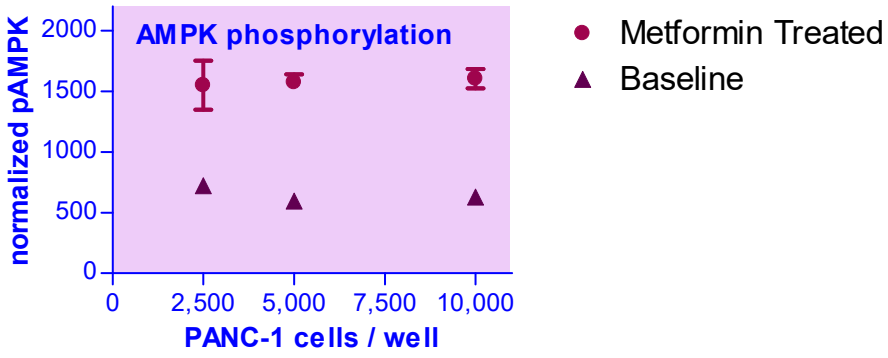
Summary

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

Results

Cell Titration

A pilot experiment was first run to determine the best cell density to plate the cells at. This example uses the cell line PANC-1. In a sterile, black 96-well plate, PANC-1 cells were plated at cell densities of 2,500, 5,000, and 10,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with medium dosed with 25mM Metformin or control medium. The dosed culture medium was prepared from a stock solution of Metformin in DMSO so that the final concentration of DMSO in the medium was 1%. Triplicate wells were incubated for 3 hours with the Metformin or control medium. The pAMPK and total protein was then quantified using the AMPK Phosphorylation Assay Kit (EAMPK-100). The pAMPK values were normalized to the total protein content of the sample. No significant difference was seen between the different cell densities.



Phosphorylation Titration

Before determining the time course of AMPK phosphorylation, a titration was run on the concentration of the test compound; here we use Metformin as an example. In a sterile, black 96-well plate, PANC-1 cells were plated at a cell density of 5,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with medium dosed with Metformin at the following concentrations: 0, 1.6E0, 8.0E0, 4.0E+1, 2.0E+2, 1.0E+3, 5.0E+3, and 2.5E+4 µM. The dosed culture

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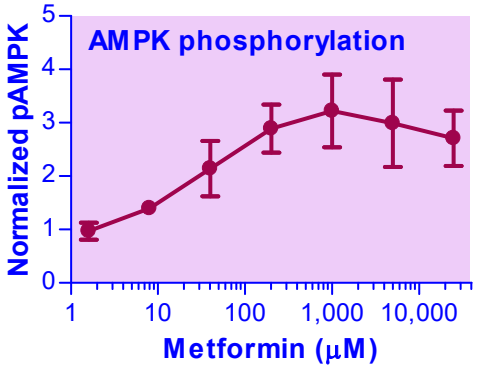
medium was prepared from a stock solution of Metformin in DMSO so that the final concentration of DMSO in the medium was 1%. Triplicate wells were incubated for 3 hours with the Metformin. The pAMPK and total protein was then quantified using the AMPK Phosphorylation Assay Kit (EAMPK-100). The pAMPK values were normalized to the total protein content of the sample.



Phosphorylation Kinetics

After the titration on the test compound concentration, we measure the AMPK phosphorylation kinetics for a specific dosage. Metformin will continue to serve as our example here; we measure the AMPK phosphorylation kinetics for Metformin at a concentration of 25 mM. In a sterile, black 96-well plate, PANC-1 cells were plated at a cell density of 5,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with 25 mM Metformin dosed medium. The dosed culture medium was prepared from a stock solution of Metformin in DMSO so that the final concentration of DMSO in the medium was 1%. Triplicate wells were incubated for 0, 10, 20, 30, 45, 60, 120, and 180 minutes with the Metformin. The pAMPK and total protein was then quantified using the AMPK Phosphorylation Assay Kit (EAMPK-100). The pAMPK values were normalized to the total protein content of the sample. The 0 minutes (no Metformin incubation) served as the untreated control for basal AMPK phosphorylation.