BioAssay Systems
3191 Coprorate Place
Hayward, CA 94545. U. S. A

Tel: 510. 782. 9988
Fax: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com

Title: NFκB Phosphorylation Status Screening Service

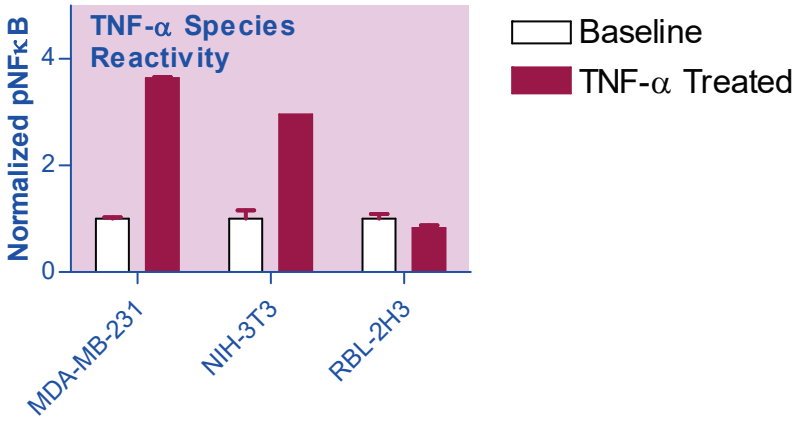
Summary

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

Results

Species Reactivity

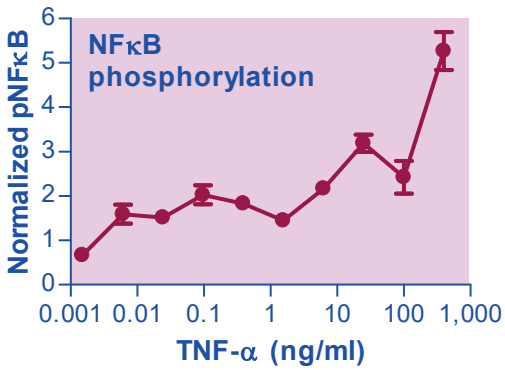
We first determine the test compound's reactivity with different species. In this example, the species tested were human (MDA-MB-231), mouse (NIH-3T3), and rat (RBL-2H3). Here we use Tumor necrosis factor alpha (TNF-α) as an example test compound. In a sterile, black 96-well plate, MDA-MB-231, NIH-3T3, and RBL-2H3 cells were plated at a cell density of 20,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with medium dosed with 10 ng/mL TNF-α or no TNF-α for the baseline controls. The dosed culture medium was prepared from a stock solution of 10,000 ng/mL TNF-α in dH_2O. Triplicate wells were incubated for 10 minutes with the TNF-α or control medium. The pNFκB and total protein was then quantified using the NFκB Phosphorylation Assay Kit (EFNKB-100). The pNFκB values were normalized to the total protein content of the sample. The relative increase in pNFκB for the cells treated with TNF-α was 3.64:1, 2.96:1, and 0.83:1 for MDA-MB-231, NIH-3T3, and RBL-2H3 respectively. The cell line MDA-MB-231 showed the greatest reactivity to the test compound TNF-α and was chosen as the cell line for the next experiments.



Phosphorylation Titration

To determine the time course of NFκB phosphorylation, first a titration was run on the concentration of the test compound; here we use TNF-α as an example. In a sterile, black 96-well plate, MDA-MB-231

cells were plated at a cell density of 20,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with medium dosed with TNF-α at the following concentrations: 0.0E+0, 3.8E-4, 1.5E-3, 6.1E-3, 2.4E-2, 9.8E-2, 3.9E-1, 1.6E+0, 6.3E+0, 2.5E+1, 1.0E+2, and 4.0E+2 ng/mL. The dosed culture medium was prepared from a stock solution of 10,000 ng/mL TNF-α in dH_2O. Triplicate wells were incubated for 20 minutes with the TNF-α. The pNFκB and total protein was then quantified using the NFκB Phosphorylation Assay Kit (ENFKB-100). The pNFκB values were normalized to the total protein content of the sample.



Phosphorylation Kinetics

After the titration on the test compound concentration, we measure the NFκB phosphorylation kinetics for a specific dosage. TNF-α will continue to serve as our example here; we measure the NFκB phosphorylation kinetics for TNF-α at a concentration of 100 ng/mL. In a sterile, black 96-well plate, PANC-1 cells were plated at a cell density of 20,000 cells per well and allowed to adhere overnight. The next day, the culture medium was removed and replaced with 100 ng/mL TNF-α dosed medium. The dosed culture medium was prepared from a stock solution of 10,000 ng/mL TNF-α in dH_2O. Triplicate wells were incubated for 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, and 120 minutes with the TNF-α. The pNFκB and total protein was then quantified using the NFκB Phosphorylation Assay Kit (ENFKB-100). The pNFκB values were normalized to the total protein content of the sample. The 0 minutes (no TNF-α incubation) served as the untreated control for basal NFκB phosphorylation.