AlphaLISA® *SureFire® Ultra™*

Human and Mouse p-Rb (Thr821) Detection Kit

Product number: ALSU-PRB-D500, ALSU-PRB-D10K, ALSU-PRB-D50K, ALSU-PRB-D-HV

Kit specificity:

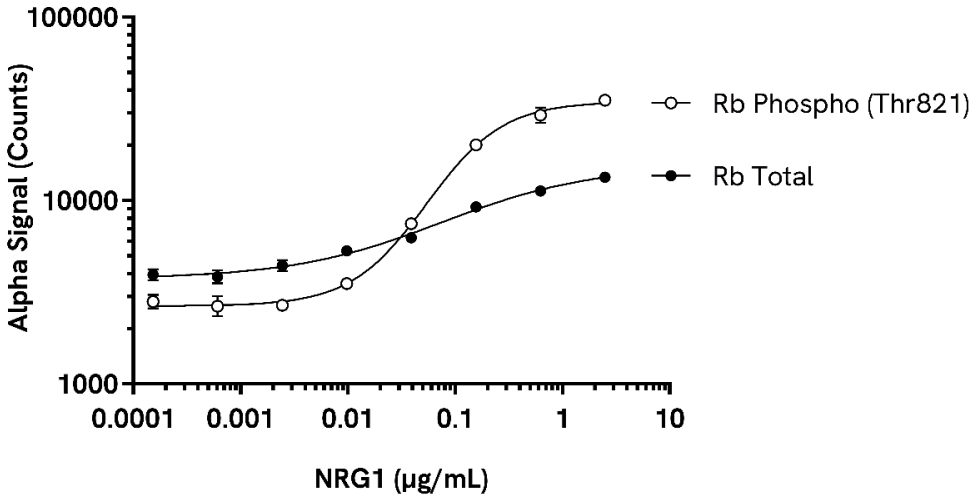
This assay kit contains antibodies which recognize phospho Thr821 epitope and a distal epitope on retinoblastoma tumor suppressor protein (Rb). The protein detected by this kit corresponds to UniProt ID P06400. Rb is also known as p105-Rb and p110-RB1. These antibodies recognize Rb of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

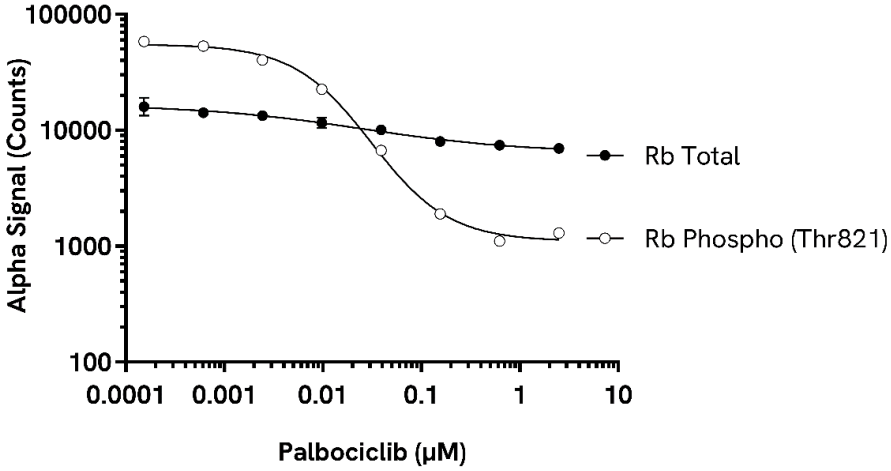
Positive Control Lysate: Prepared from Jurkat cells cultured in 10% FBS containing media. Cells were harvested and washed with HBSS + 0.1% BSA, adjusted to 4 x 10^6 cells/mL and lysed with the addition of 5X Lysis Buffer.

Representative data:

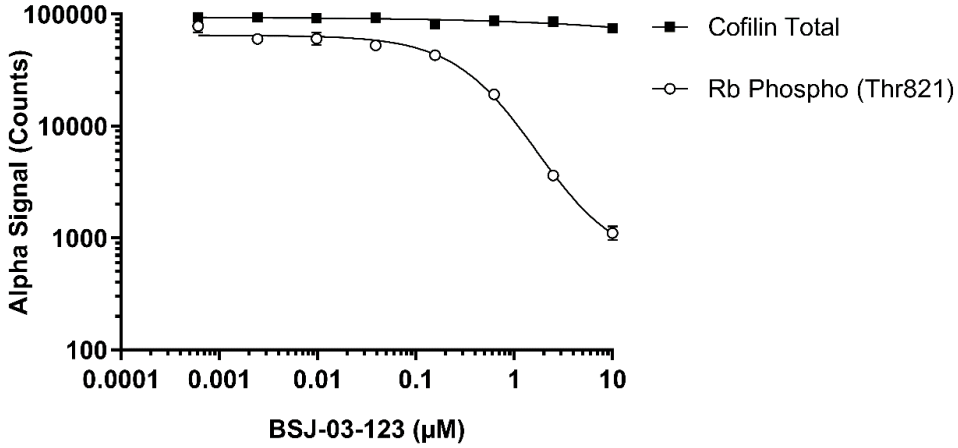
Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were plated at 40K cells/well in a 96 well plate and incubated overnight. Cells were pretreated with 20 µM Wortmannin for 2 hours then treated with NRG1 at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr821) and Total Rb using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were plated at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with Palbociclib at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr821) and Total Rb using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were plated at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with BSJ-03-123 (CDK6 PROTAC) at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Rb Phospho (Thr821) and Total Cofilin using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint or 80 cells/datapoints for Total Cofilin.



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