AlphaLISA® *SureFire® Ultra™*

Human and Mouse AKT3 Total Detection Kit

Product number: ALSU-TAKT3-A 500, ALSU-TAKT3-A 10K, ALSU-TAKT3-A 50K, ALSU-TAKT3-A-HV

Kit specificity:

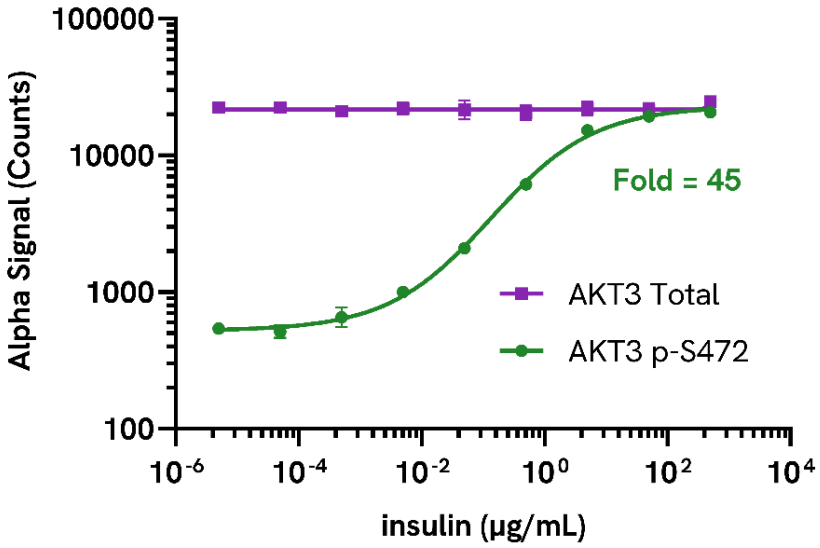
This assay kit contains antibodies which recognize distinct epitopes on AKT3. The protein detected by this kit corresponds to UniProt ID Q9Y243. AKT3 is also known as RAC-gamma serine/threonine-protein kinase or PKB gamma. These antibodies recognize AKT3 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

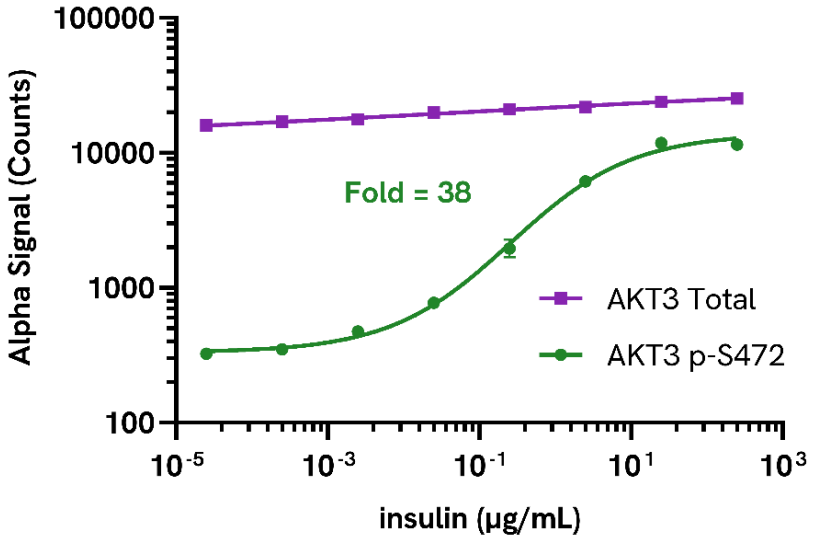
Positive Control Lysate: Prepared from HEK293 cells, cultured to confluence in T175 flasks in 10 % FBS containing medium, then treated with 20 % FBS for 15 minutes and lysed with 4 mL of Lysis Buffer

Representative data:

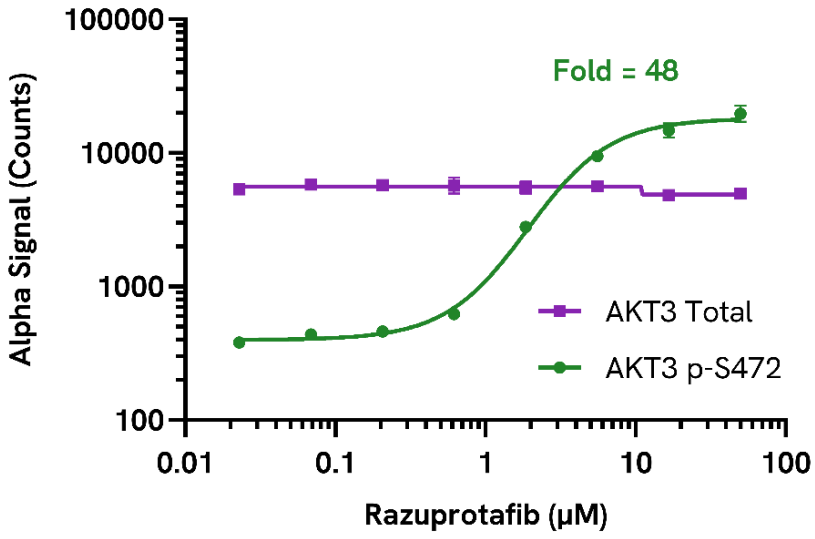
Data obtained with a 2-plate, 2-incubation protocol. SH-SY5Y cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with Insulin at the indicated concentrations for 30 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser472) and Total AKT3 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



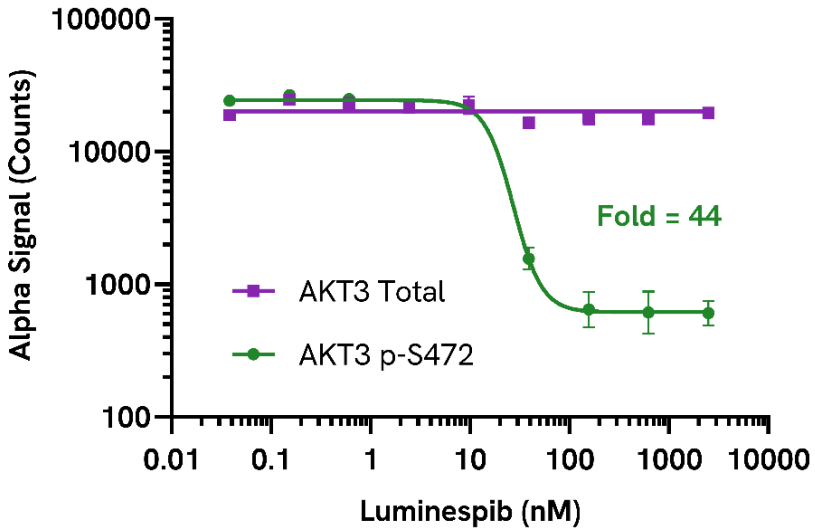
Data obtained with a 2-plate, 2-incubation protocol. NIH/3T3 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with Insulin at the indicated concentrations for 30 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser472) and Total AKT3 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



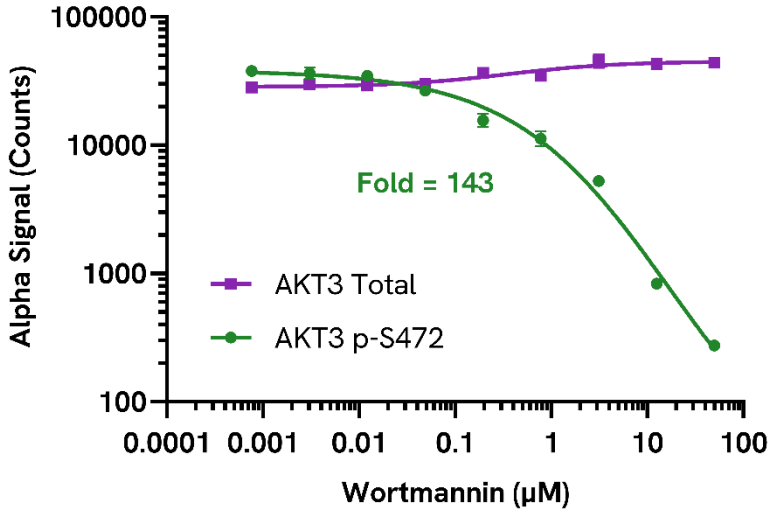
Data obtained with a 2-plate, 2-incubation protocol. HUVEC cells were seeded at 20K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with Razuprotafib at the indicated concentrations for 15 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser472) and Total AKT3 using respective *SureFire Ultra* kits. Equivalent to approximately 2,000 cells/datapoint.



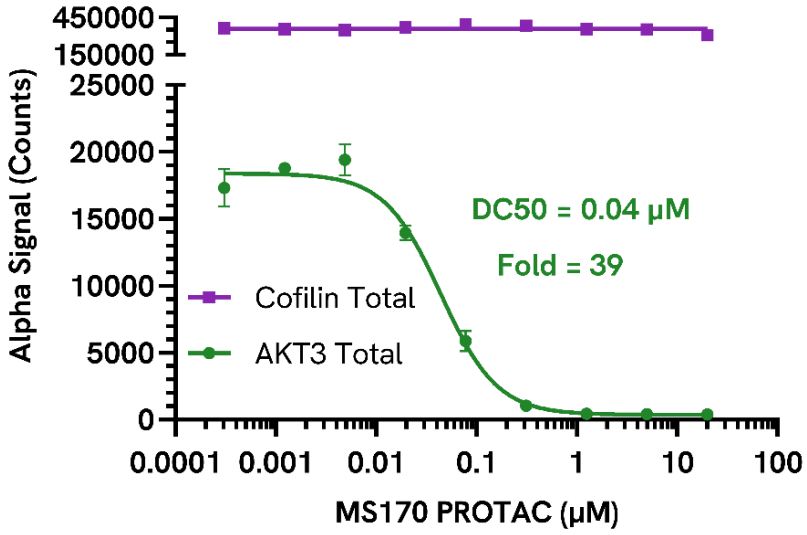
Data obtained with a 2-plate, 2-incubation protocol. HEK293 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with Luminespib at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser472) and Total AKT3 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



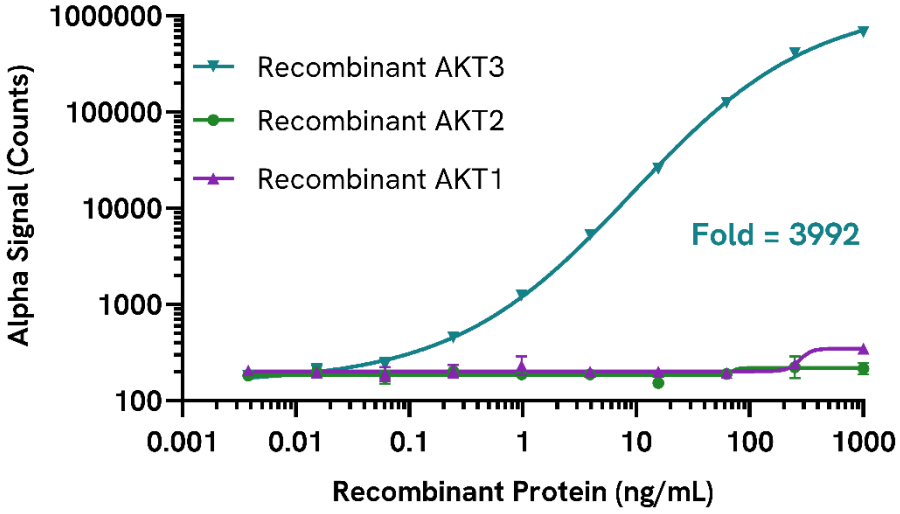
Data obtained with a 2-plate, 2-incubation protocol. HEK293 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with Wortmannin at the indicated concentrations for 2 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser472) and Total AKT3 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. PC3 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with MC170 PROTAC at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Total AKT3 and Cofilin using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



AKT1 (Abcam, ab62279), AKT2 (Abcam, ab60324) and AKT3 (Abcam, ab60324) active recombinant human proteins were serially diluted with Lysis Buffer and evaluated using the AKT3 Total *SureFire Ultra* kit. No cross-reactivity against AKT1 or AKT2 was observed despite sharing approximately 80% similarity with AKT3.



Data obtained from measurement of AKT3 in various cell types lysed with Lysis Buffer. Equivalent to approximately 4,000 cells/datapoint (adherent cells) or 40,000 cells/datapoint (suspension cells).