

AlphaLISA® SureFire® Ultra™

Human and Mouse p-AKT2 (Ser474) Detection Kit

Product number: ALSU-PAKT2-A500, ALSU-PAKT2-A10K,
ALSU-PAKT2-A50K, ALSU-PAKT2-A-HV



Kit specificity:

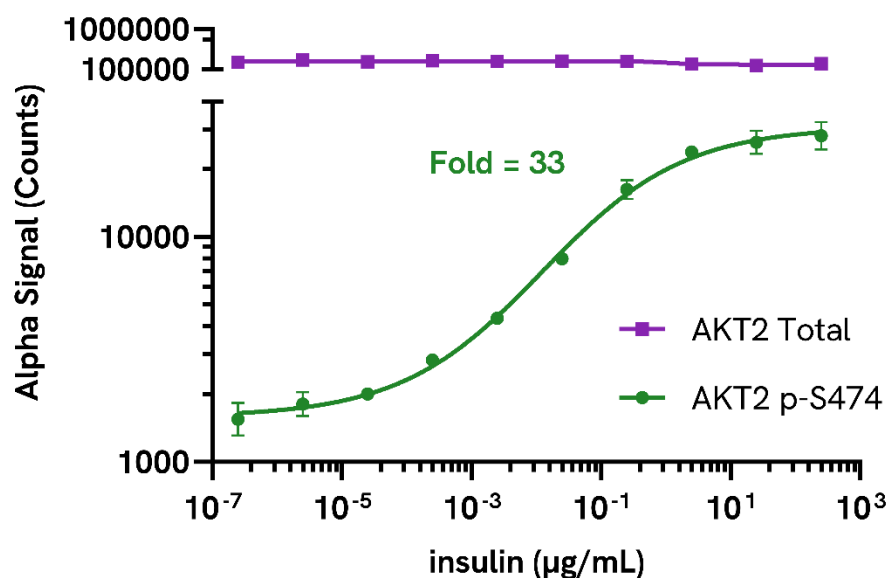
This assay kit contains antibodies which recognize the phospho-Ser474 epitope and a distal epitope on AKT2. The protein detected by this kit corresponds to UniProt ID P31751. AKT2 is also known as RAC-beta serine/threonine-protein kinase or PKB beta. These antibodies recognize AKT2 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

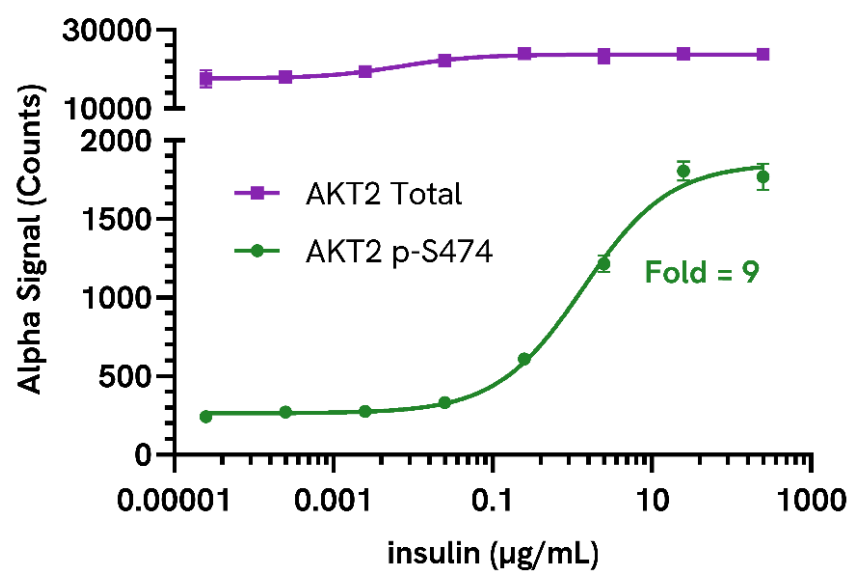
Positive Control Lysate: Prepared from HEK293 cells, cultured to confluence in T175 flasks in 10% FBS containing medium, then treated with 20% FBS for 15 minutes and lysed with 4 mL of Lysis Buffer.

Representative data:

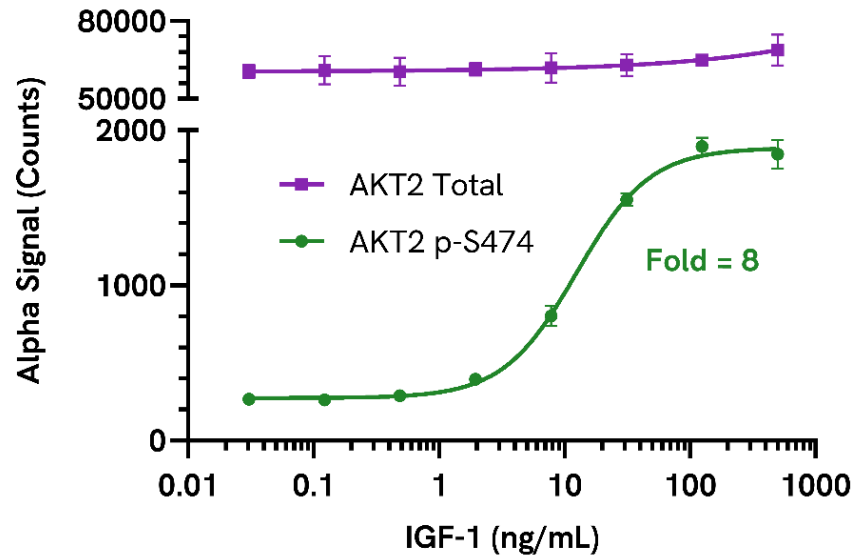
Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with insulin at the indicated concentrations for 30 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser474) and Total AKT2 using respective SureFire Ultra kits. Equivalent to approximately 4,000 cells/datapoint.



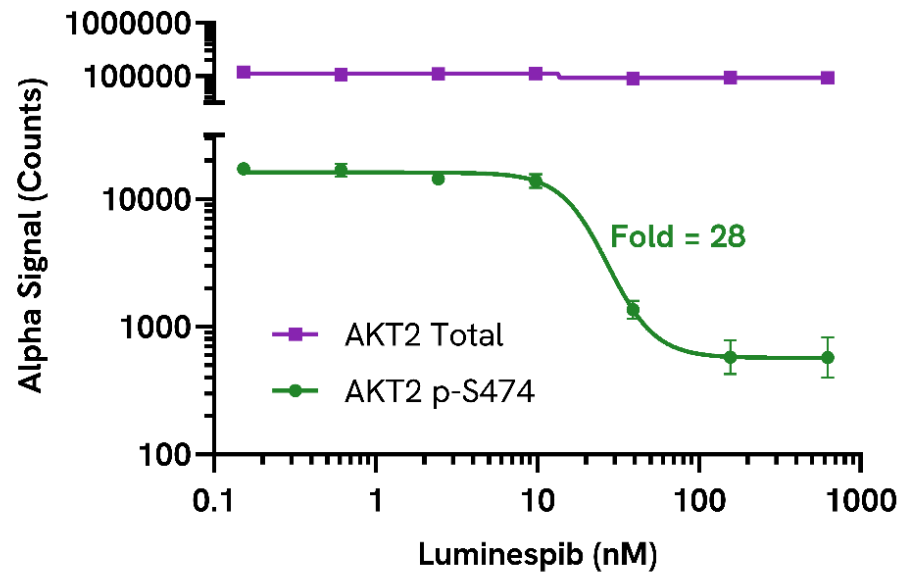
Data obtained with a 2-plate, 2-incubation protocol. NIH/3T3 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with insulin at the indicated concentrations for 30 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser474) and Total AKT2 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



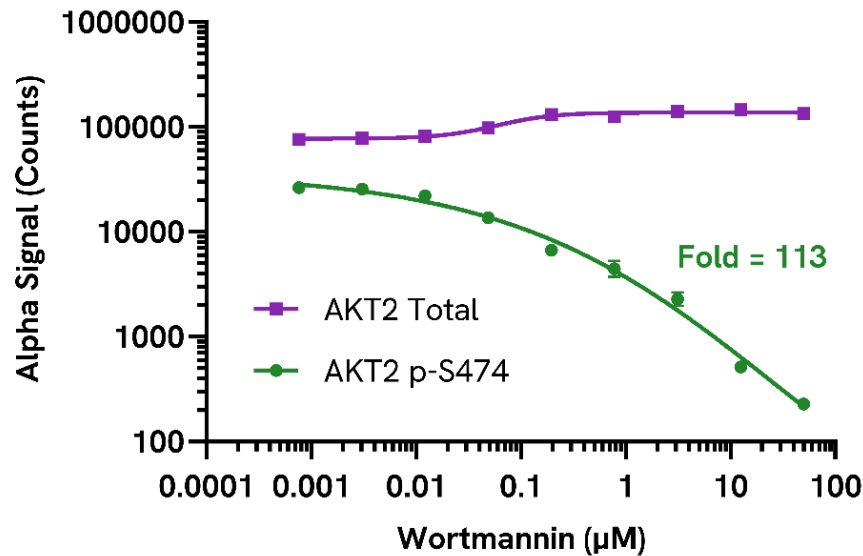
Data obtained with a 2-plate, 2-incubation protocol. HeLa cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were serum starved for 2 hours, then treated with IGF-1 at the indicated concentrations for 15 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser474) and Total AKT2 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



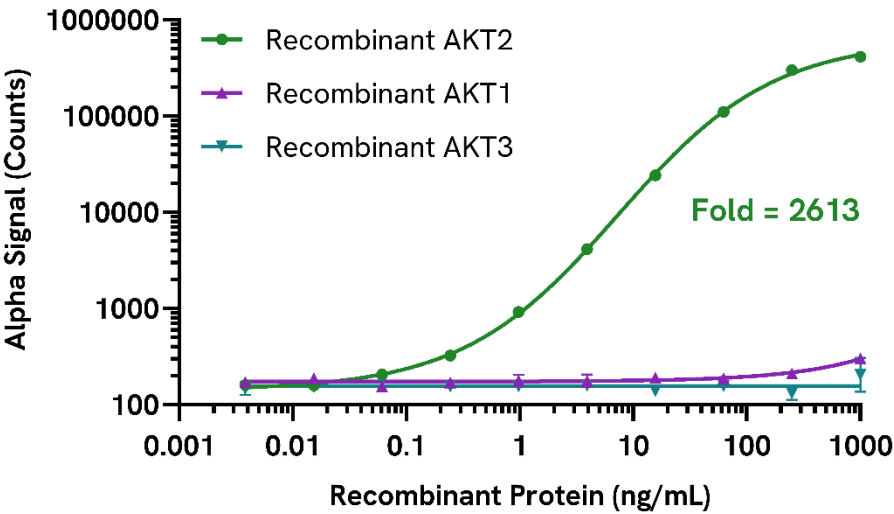
Data obtained with a 2-plate, 2-incubation protocol. HEK293 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with Luminespib at the indicated concentrations for 24 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser474) and Total AKT2 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 (Ser474) and Total AKT2 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 AKT2 Total *SureFire Ultra* kit. No cross-reactivity against AKT1 or AKT3 was observed despite sharing approximately 80% similarity with AKT2.



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