AlphaLISA® *SureFire® Ultra™*

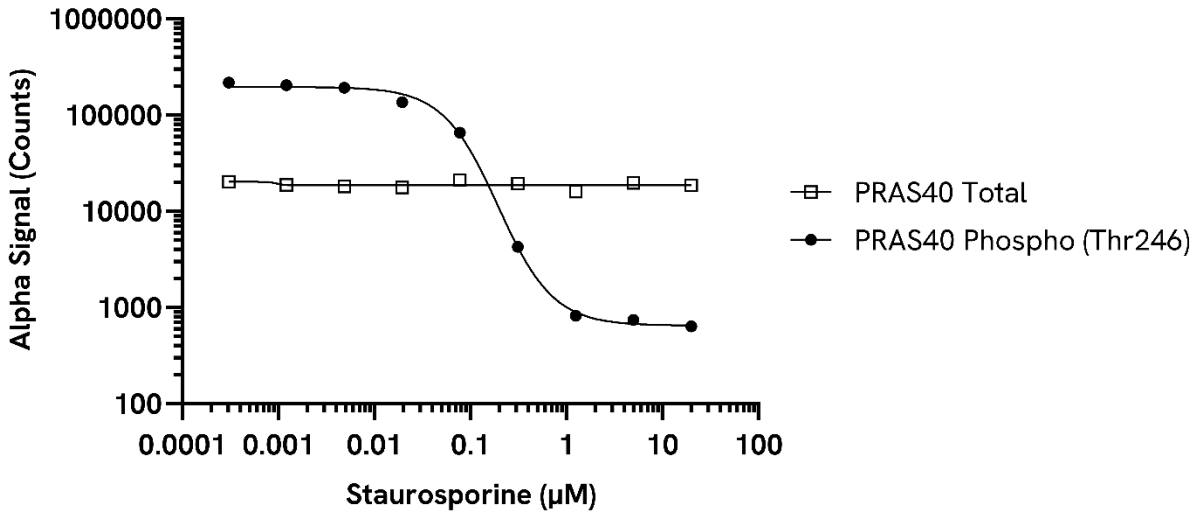
Human and Mouse p-PRAS40 (Thr246) Detection Kit

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

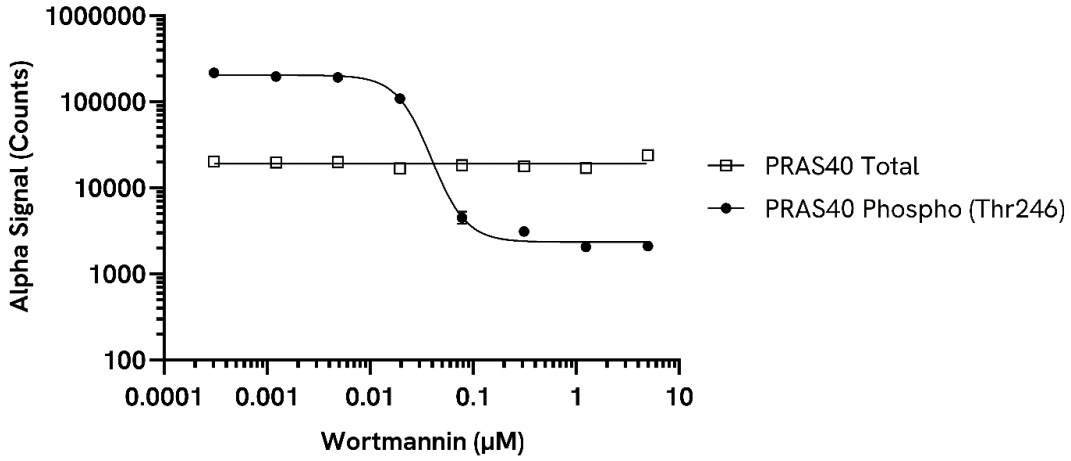
Kit specificity:
This assay kit contains antibodies which recognize phospho Thr246 epitope and a distal epitope on PRAS40. The protein detected by this kit corresponds to UniProt ID Q96B36. PRAS40 is also known as proline-rich AKT1 substrate1. These antibodies recognize PRAS40 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:
Positive Control Lysate: Prepared from A549 cells, cultured to confluence in T175 flasks in 10% FBS containing medium and lysed with 4 mL of Lysis Buffer.

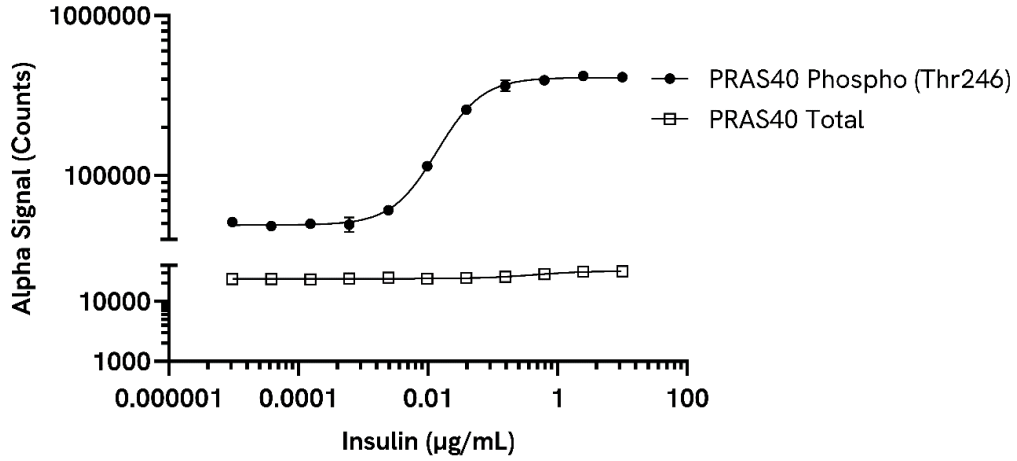
Representative data:
Data obtained with a 2-plate, 2-incubation protocol. A549 cells were seeded at 40K cells/well in a 96 well plate and incubated overnight. Cells were treated with Staurosporine at the indicated concentrations for 1 hour. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr246) and Total PRAS40 using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. A549 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 1 hour. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr246) and Total PRAS40 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 20K cells/well in a 96 well plate and incubated over two nights. Cells were starved for 3 hours and then treated with Insulin at the indicated concentrations for 5 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr246) and Total PRAS40 using respective *SureFire Ultra* kits. Equivalent to approximately 2,000 cells/datapoint



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