AlphaLISA® *SureFire® Ultra™*

Human and Mouse p-Paxillin (Tyr118) Detection Kit

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

Kit specificity:

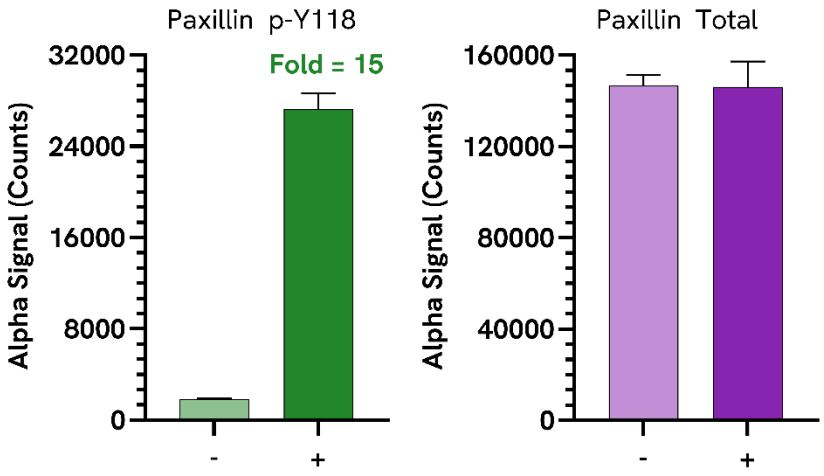
This assay kit contains antibodies which recognize the phospho-Tyr118 epitope and a distal epitope on Paxillin. The protein detected by this kit corresponds to UniProt ID P49023. Paxillin is also known as PXN. These antibodies recognize Paxillin of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

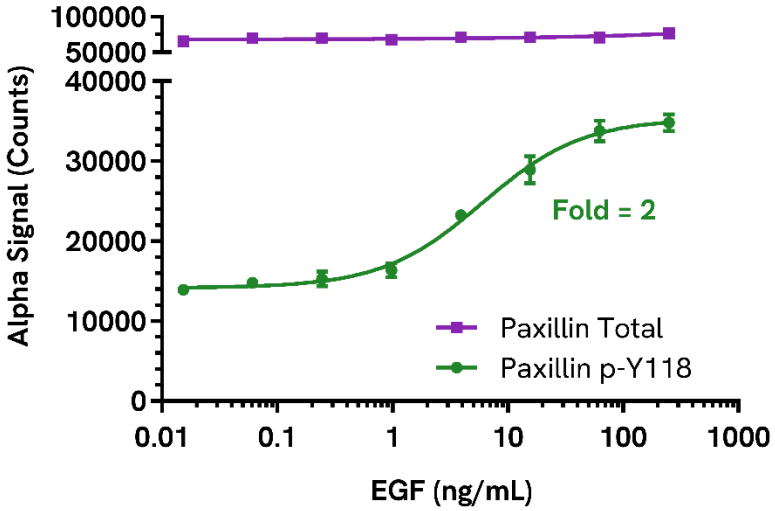
Positive Control Lysate: Prepared from A431 cells, cultured to confluence in T175 flasks in 10% FBS containing medium and lysed with 5 mL of Lysis Buffer.

Representative data:

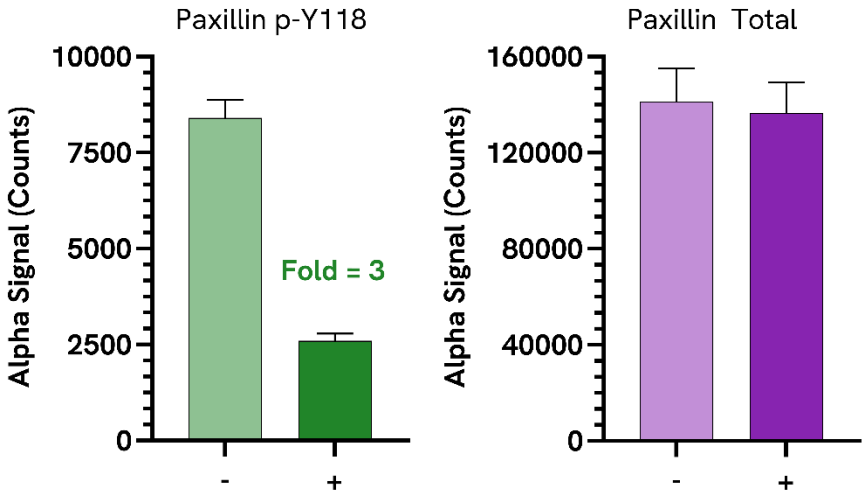
Data obtained with a 2 plate, 2 incubation protocol. T25 flasks were coated with 10µg/mL Fibronectin prepared in PBS and incubated overnight at 4°C. HeLa cells were seeded at 1.4 x 10^6 cells/T25 flask and incubated for 1 hour. Cells were lysed with 5X Lysis Buffer and assayed separately for Phospho (Tyr118) and Total Paxillin using respective *SureFire Ultra* kits. Equivalent to approximately 10,000 cells/datapoint.



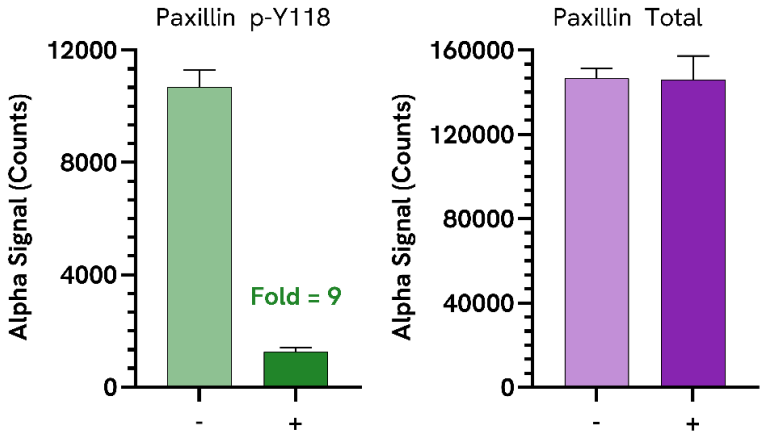
Data obtained with a 2 plate, 2 incubation protocol. HeLa cells were seeded at 20K cells/well in a 96 well plate and incubated overnight. Cells were treated with EGF at the indicated concentrations for 15 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr118) and Total Paxillin using respective *SureFire Ultra* kits. Equivalent to approximately 4,000 cells/datapoint.



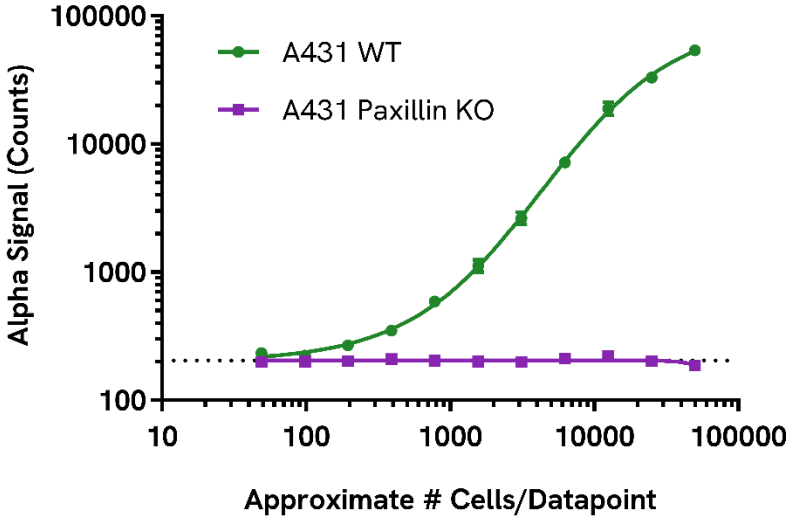
Data obtained with a 2 plate, 2 incubation protocol. PANC-1 cells were seeded at 30K cells/well in a 96 well plate and incubated overnight. Cells were untreated or treated with 50 µM Defactinib for 3 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr118) and Total Paxillin using respective *SureFire Ultra* kits. For Total Paxillin, lysates were further diluted to work within the assay linear range. Equivalent to approximately 3,000 cells/datapoint (Phospho Paxillin) or 750 cells/datapoint (Total Paxillin).



Data obtained with a 2 plate, 2 incubation protocol. PANC-1 cells were seeded at 30K cells/well in a 96 well plate and incubated overnight. Cells were untreated or treated with 50 µM PF-562271 for 2 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr118) and Total Paxillin using respective *SureFire Ultra* kits. For Total Paxillin, lysates were further diluted to work within the assay linear range. Equivalent to approximately 3,000 cells/datapoint (Phospho Paxillin) or 750 cells/datapoint (Total Paxillin).



Data obtained with a 2-plate, 2-incubation protocol. A431 wild type (WT) and A431 Paxillin knockout (KO, Abcam ab261892) cell lines were seeded at increasing cell densities in a 96 well plate and incubated overnight. Cells were lysed with Lysis Buffer and assayed for Phospho (Tyr118) Paxillin using the *SureFire Ultra* kit.



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