AlphaLISA® *SureFire® Ultra™*

Human p-IRF5 (Ser446) Detection Kit

Product number: ALSU-PIRF5-A-500, ALSU-PIRF5-A10K, ALSU-PIRF5-A50K, ALSU-PIRF5-A-HV

Kit specificity:

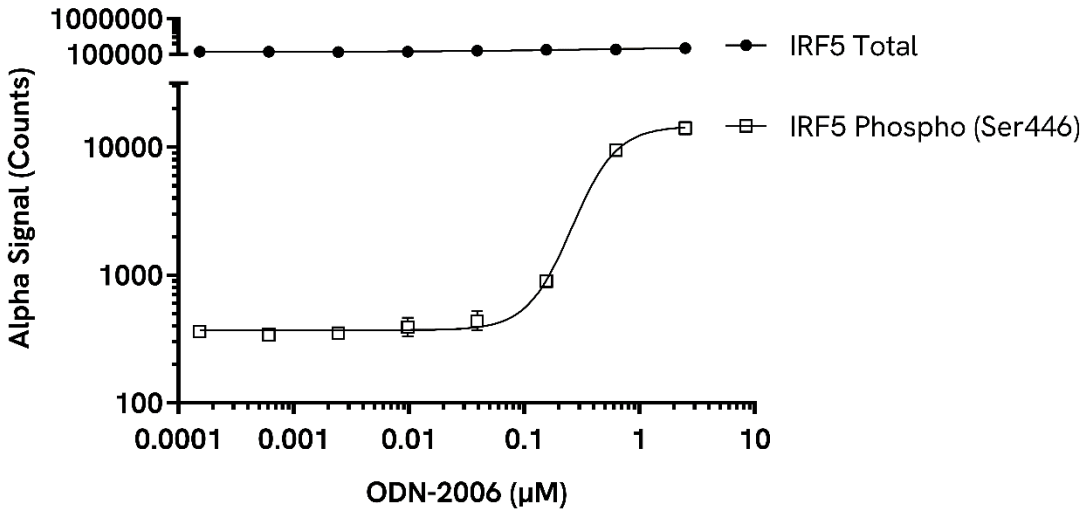
This assay kit contains antibodies which recognize the phospho Ser-446 epitope and a distal epitope on Interferon regulatory factor 5 (IRF5). The protein detected by this kit corresponds to UniProt ID Q13568. These antibodies recognize IRF5 of human origin. Other species should be tested on a case-by-case basis.

Control lysate information:

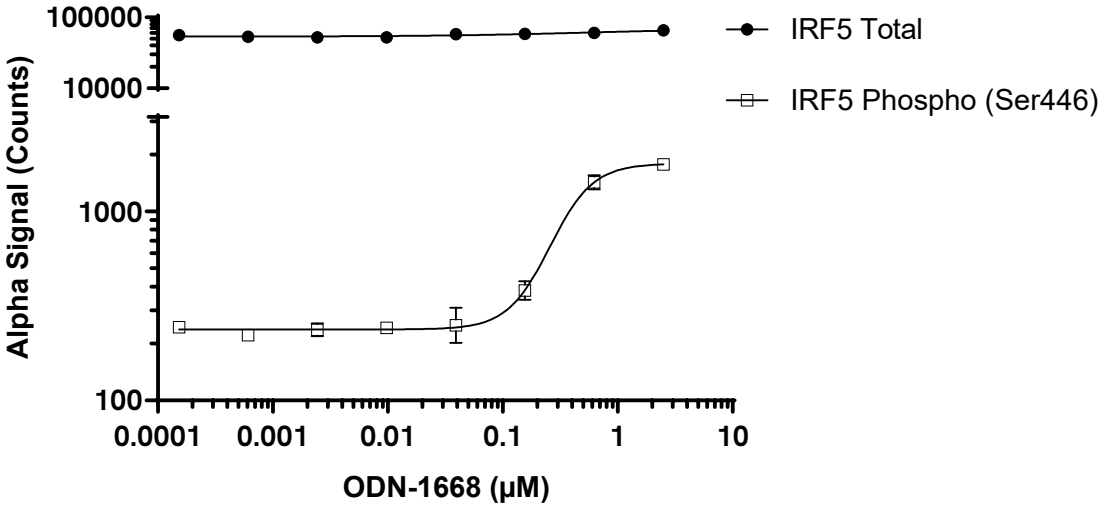
Positive Control Lysate: Prepared from THP-1 cells, seeded at 0.3x10^6 cells/mL and incubated for 48 hours in T175 flasks in 10% FBS containing medium. Cells were harvested, resuspended at 5x10^6 cells/mL and then treated with 100 nM Calyculin A for 3 hours. Cells were washed in HBSS + 0.1% BSA and lysed in Lysis Buffer at a final cell density of 8x10^6 cells/mL.

Representative data:

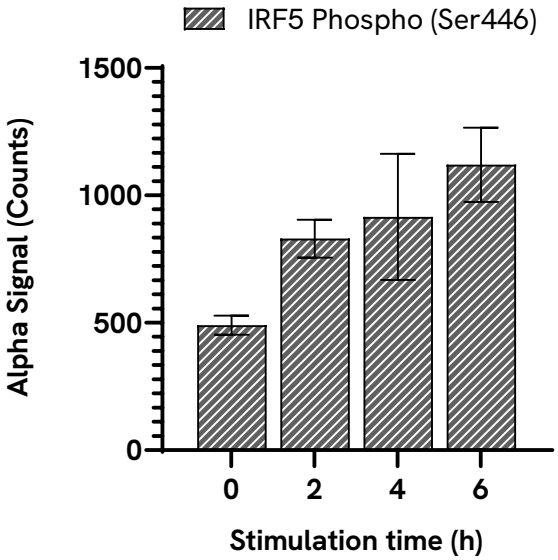
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 500K cells/well in a 96 well plate. Cells were treated with CpG ODN-2006 at the indicated concentrations for 6 hours. After treatment, cells washed in HBSS + 0.1% BSA and lysed with Lysis Buffer. Cell lysates were assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire Ultra* kits. Equivalent to approximately 50,000 cells/datapoint.



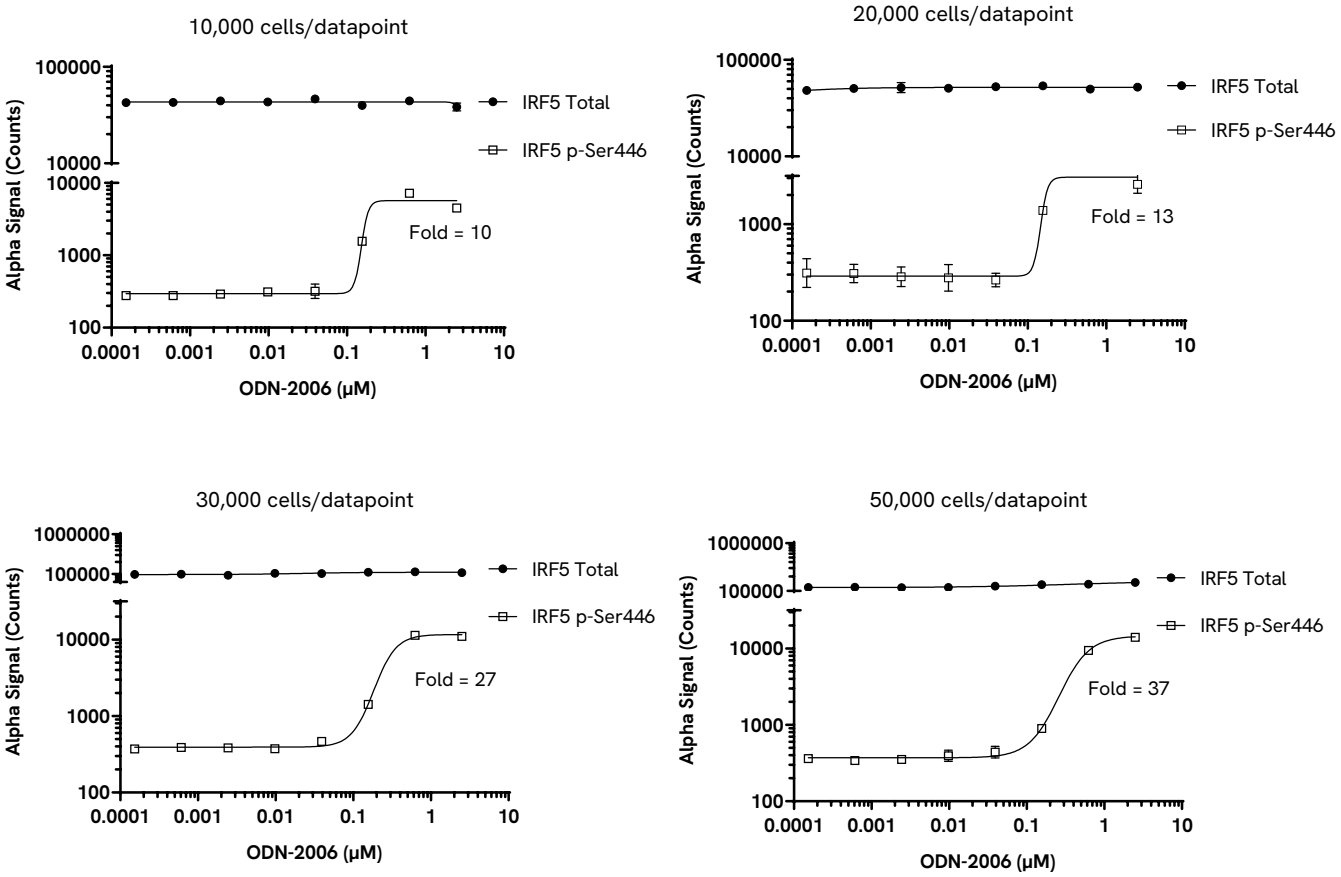
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 200K cells/well in a 96 well plate. Cells were treated with CpG ODN-1668 at the indicated concentrations for 6 hours. After treatment, cells were washed in HBSS + 0.1% BSA and lysed with Lysis Buffer. Cell lysates were assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire Ultra* kits. Equivalent to approximately 20,000 cells/datapoint.



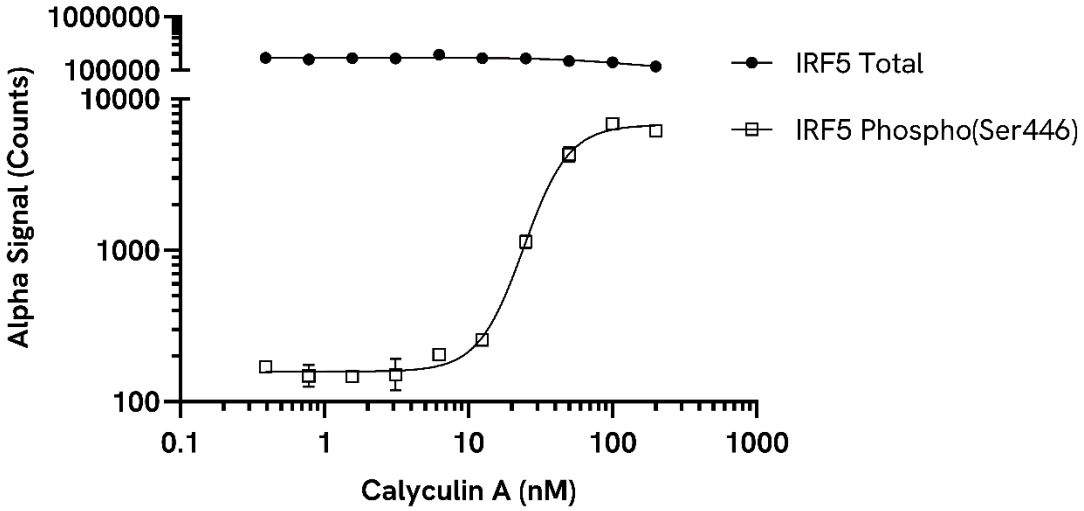
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 200K cells/well in a 96 well plate. Cells were treated with 1 µM Resiquimod (R848) at the indicated timepoints. After time course, cells were washed in HBSS + 0.1% BSA and lysed with Lysis Buffer. Cell lysates were assayed for IRF5 Phospho (Ser446) using *SureFire Ultra*. Equivalent to approximately 20,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at various densities in a 96 well plate. Cells were treated with CpG ODN-2006 at the indicated concentrations for 6 hours. After treatment, cells were washed in HBSS + 0.1% BSA and lysed with Lysis Buffer. Cell lysates were assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire Ultra* kits. Approximate number of cells/datapoint is outlined above each graph.



Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 400K cells/well in a 96 well plate. Cells were then treated with Calyculin A at the indicated concentrations for 3 hours. Cells were washed in HBSS + 0.1% BSA and lysed with Lysis Buffer. Cell lysates were assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire Ultra* kits. Equivalent to approximately 40,000 cells/datapoint.



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