AlphaLISA™ *SureFire*® Biotin Free

Human and Mouse IRF5 Total Detection Kit

Product number: ASBF-TIRF5-A500, ASBF-TIRF5-A10K, ASBF-TIRF5-A50K, ASBF-TIRF5-A-HV

Kit specificity:

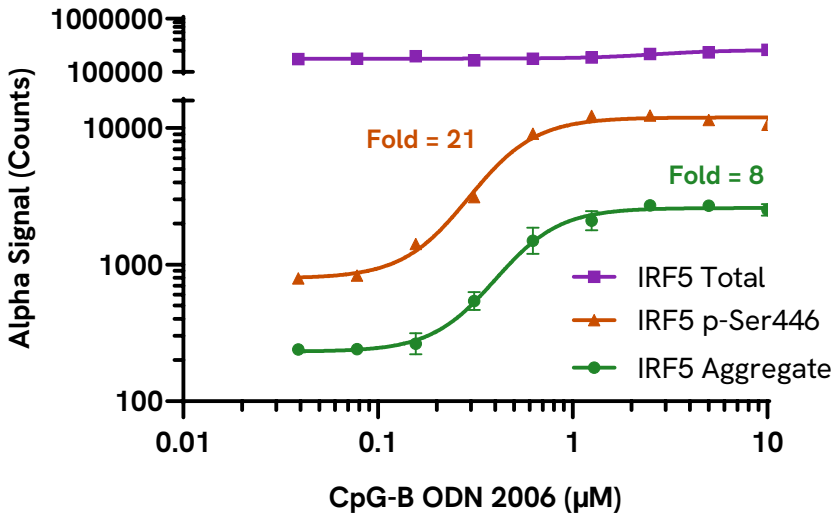
This assay kit contains antibodies which recognize distinct epitopes on IRF5. The protein detected by this kit corresponds to UniProt ID Q13568. IRF5 is also known as Interferon regulatory factor 5. These antibodies recognize IRF5 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

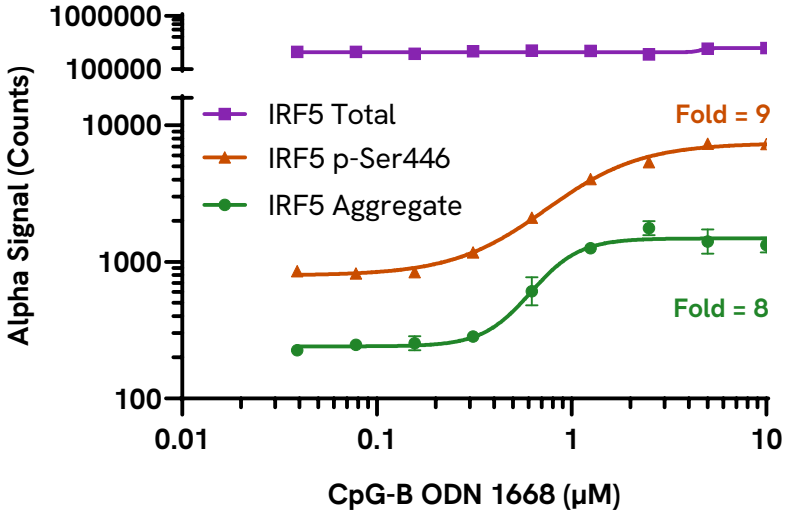
Positive Control Lysate: Prepared from THP-1 cells, seeded at 0.3 x 10^6 cells/mL, and incubated for 48 hours in T175 flasks in 10% FBS containing medium. Cells were harvested and resuspended at 8 x 10^6 cells/mL and treated with 100 nM Calyculin A for 3 hours. Cells were then washed in HBSS + 0.1% BSA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

Representative data:

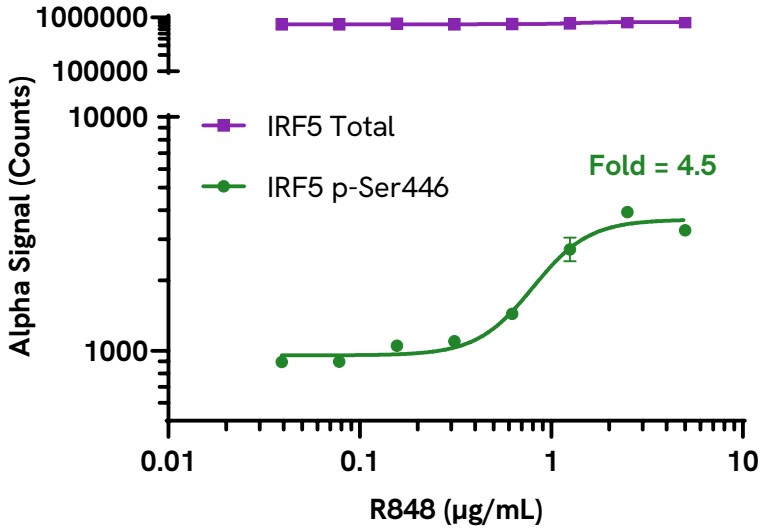
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 200K/well in a 96-well plate. Cells were then treated with CpG-B ODN 2006 at the indicated concentrations for 3 hours. Cells were spun down at 1200 RPM for 5 minutes, supernatant was removed and cells were lysed with Lysis Buffer and assayed separately for Aggregate, Phospho (Ser446) and Total IRF5 using respective *SureFire* Biotin Free kits. Equivalent to approximately 40,000 cells/datapoint.



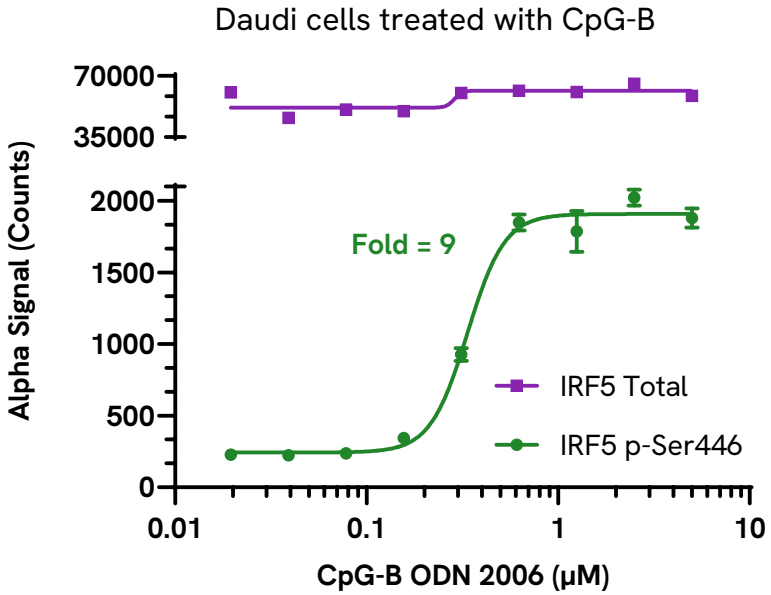
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 200K/well in a 96-well plate. Cells were then treated with CpG-B ODN 1668 at the indicated concentrations for 3 hours. Cells were spun down at 1200 RPM for 5 minutes, supernatant was removed and cells were lysed with Lysis Buffer and assayed separately for Aggregate, Phospho (Ser446) and Total IRF5 using respective *SureFire* Biotin Free kits. Equivalent to approximately 40,000 cells/datapoint.



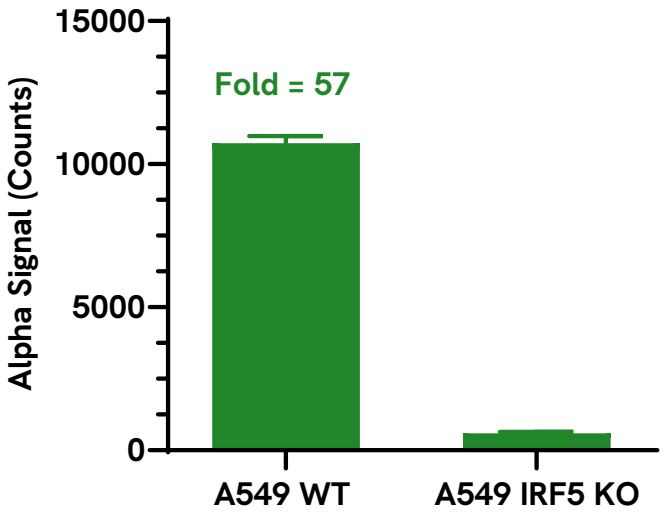
Data obtained with a 2-plate, 2-incubation protocol. RPMI 8226 cells were seeded at 400K/well in a 96-well plate. Cells were then treated with R848 at the indicated concentrations for 4 hours. Cells were spun down at 1200 RPM for 5 minutes, supernatant was removed and cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire* Biotin Free kits. Equivalent to approximately 80,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. Daudi cells were seeded at 400K/well in a 96-well plate. Cells were then treated with CpG-B ODN 2006 at the indicated concentrations for 3 hours. Cells were spun down at 1200 RPM for 5 minutes, supernatant was removed and cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser446) and Total IRF5 using respective *SureFire* Biotin Free kits. Equivalent to approximately 80,000 cells/datapoint.



A549 wild type (WT) and A549 IRF5 knockout (KO) (Abcam, ab301006) cells were cultured to confluency in T75 flasks. Each flask was lysed in 2 mL of Lysis Buffer and lysates were evaluated for Total IRF5 using the *SureFire* Biotin Free kit. Equivalent to approximately 10,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. Adherent cell lines were seeded at 40K/well in a 96-well plate and incubated overnight. Suspension cell lines were seeded at 400K/well in HBSS + 0.1% BSA in a 96-well plate. Adherent and suspension cell lines were then lysed with Lysis Buffer, lysates were further diluted to 50% and evaluated for Total IRF5 using the *SureFire* Biotin Free kit. Equivalent to approximately 2,000 cells/datapoint for adherent cell lines and 20,000 cells/datapoint for suspension cell lines.