AlphaLISA® *SureFire® Ultra™*

# Human IRF7 Total Detection Kit

**Product number:** ALSU-TIRF7-A500, ALSU-TIRF7-A10K, ALSU-TIRF7-A50K, ALSU-TIRF7-A-HV

**Kit specificity:**

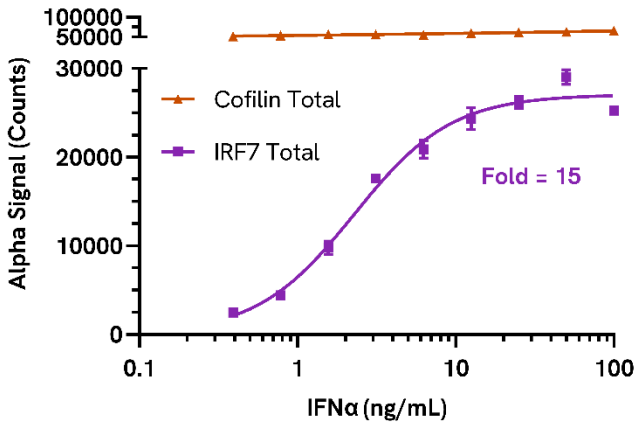
This assay kit contains antibodies which recognize distinct epitopes on IRF7. The proteins detected by this kit correspond to UniProt ID Q92985. IRF7 is also known as interferon regulatory factor 7. These antibodies recognize IRF7 of human origin. Other species should be tested on a case-by-case basis.

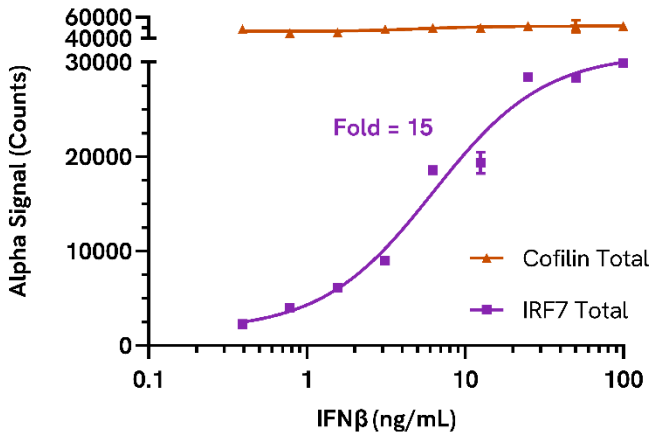
**Control lysate information:**

Positive Control Lysate: Prepared from THP-1 cells seeded at 500K cells/mL in medium containing 100 nM PMA and incubated for 24 hours. THP-1 derived macrophages were treated with 100 nM Calyculin A for 30 minutes, washed with HBSS and lysed in Lysis Buffer at a final concentration of 8 x $10^6$ cells/mL.

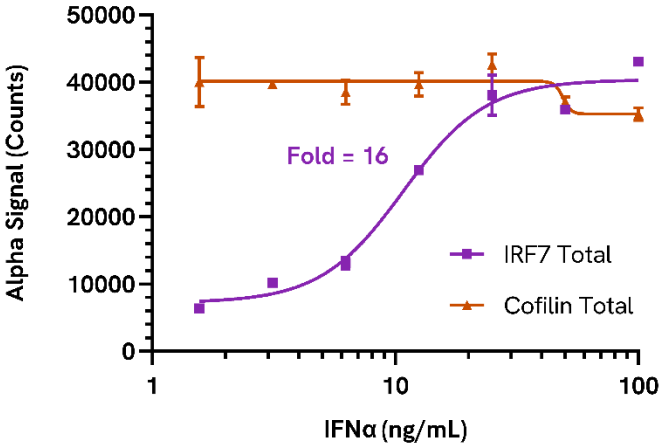
**Representative data:**

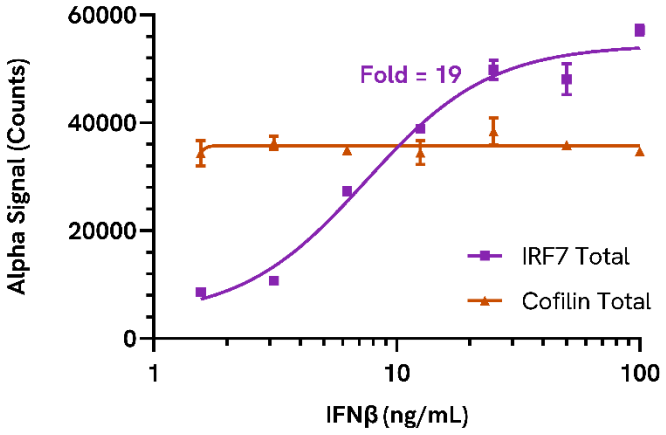
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 200K cells/well and treated with IFNα or IFNβ at the indicated concentrations for 6 hours. Cells were washed with HBSS and lysed with Lysis Buffer. Lysates were assayed separately for Total IRF7 and Cofilin Total using respective *SureFire Ultra* kits. Equivalent to approximately 20,000 cells/datapoint for Total IRF7 and 400 cells/datapoint for Total Cofilin.



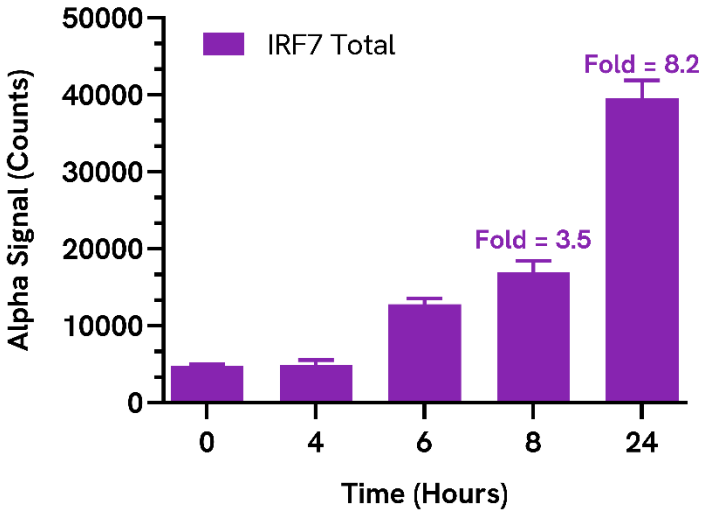



Data obtained with a 2-plate, 2-incubated protocol. HT 29 cells were seeded at 20K cells/well and incubated overnight. Cells were treated with IFNα or IFNβ at the indicated concentrations for 24 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately Total IRF7 and Cofilin Total using respective *SureFire Ultra* kits. Equivalent to approximately 1,000 cells for Total IRF7 and 40 cells for Cofilin Total.

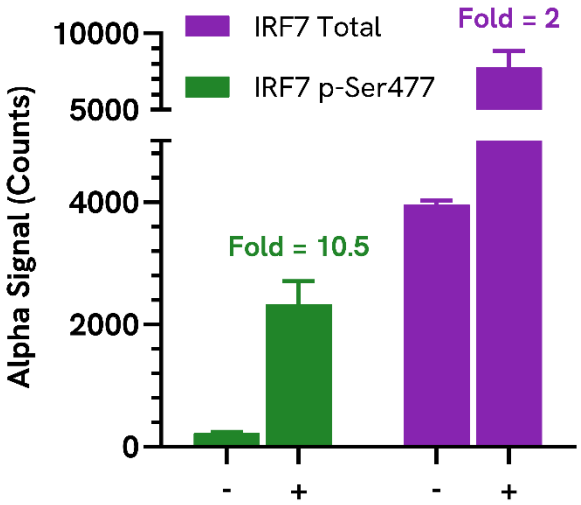


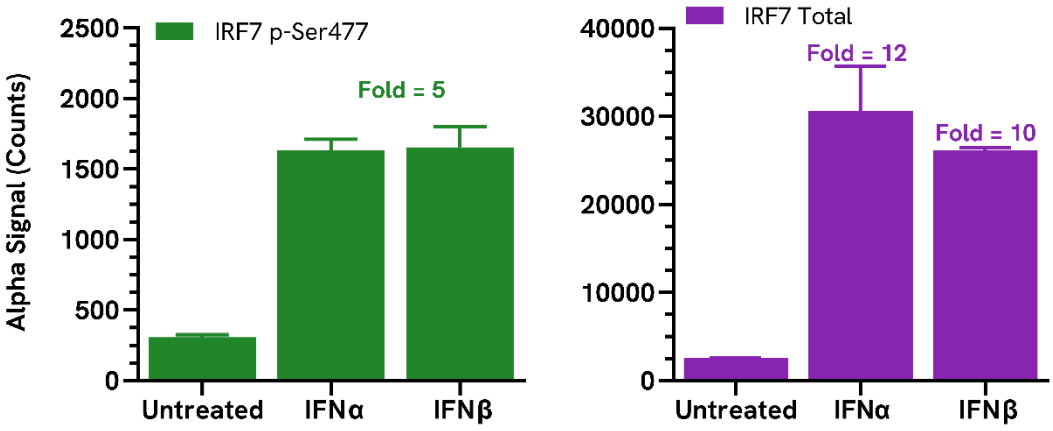

Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 400K cells/well and treated with 20 µM of STING agonist, diABZI at the indicated time points. Cells were washed with HBSS, lysed with Lysis Buffer and assayed for Total IRF7 using the *SureFire Ultra* kit. Equivalent to approximately 40,000 cells/datapoint.



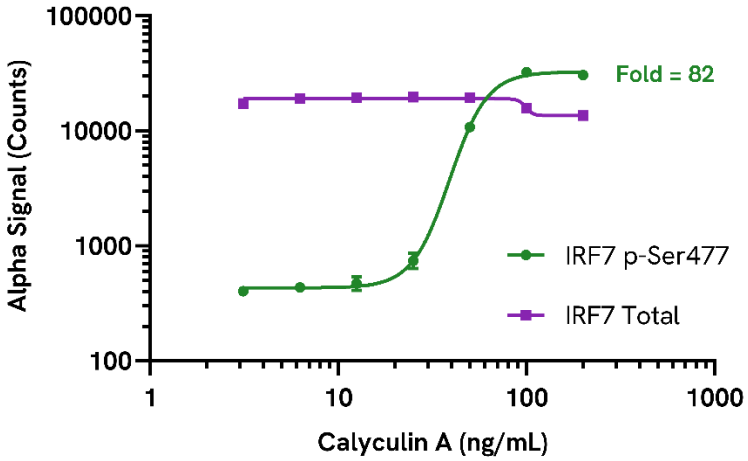
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 400K cells/well and treated with 100 µg/mL of STING ligand, 2'3' cGAMP for 4 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* kits. Equivalent to approximately 40,000 cells/datapoint.




Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 100K cells/well and incubated in medium containing 100nM PMA for 24 hours. Cells were then treated with 250 ng/mL of IFNα or IFNβ for a further 24 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* kits. Equivalent to approximately 10,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 100K cells/well in medium containing 100nM PMA and incubated for 18 hours. Cells were treated with Calyculin A at the indicated concentrations for 30 minutes. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* kits. Equivalent to approximately 10,000 cells/datapoint.




Data obtained from measurement of Total IRF7 protein levels in various cell types. Adherent cell lines were seeded at 40K cells/well and incubated overnight and lysed with Lysis Buffer. Suspension cell lines were seeded at 400K cells/well and lysed with Lysis Buffer. Suspension and adherent cell lysates were then assayed for Total IRF7 using the *SureFire Ultra* kit. Equivalent to approximately 4,000 cells/datapoint for adherent and 40,000 cells/datapoint for suspension cell lines.