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TECHNICAL DATA SHEET

Research use only. Not for use in diagnostic procedures.

AlphaLISA® *SureFire® Ultra™*

Human and Mouse p-STAT3 (Tyr705) High Performance Detection Kit

Product number: ALSU-PST3-B500, ALSU-PST3-B10K, ALSU-PST3-B50K, ALSU-PST3-B-HV

TGR BioSciences

Kit specificity:

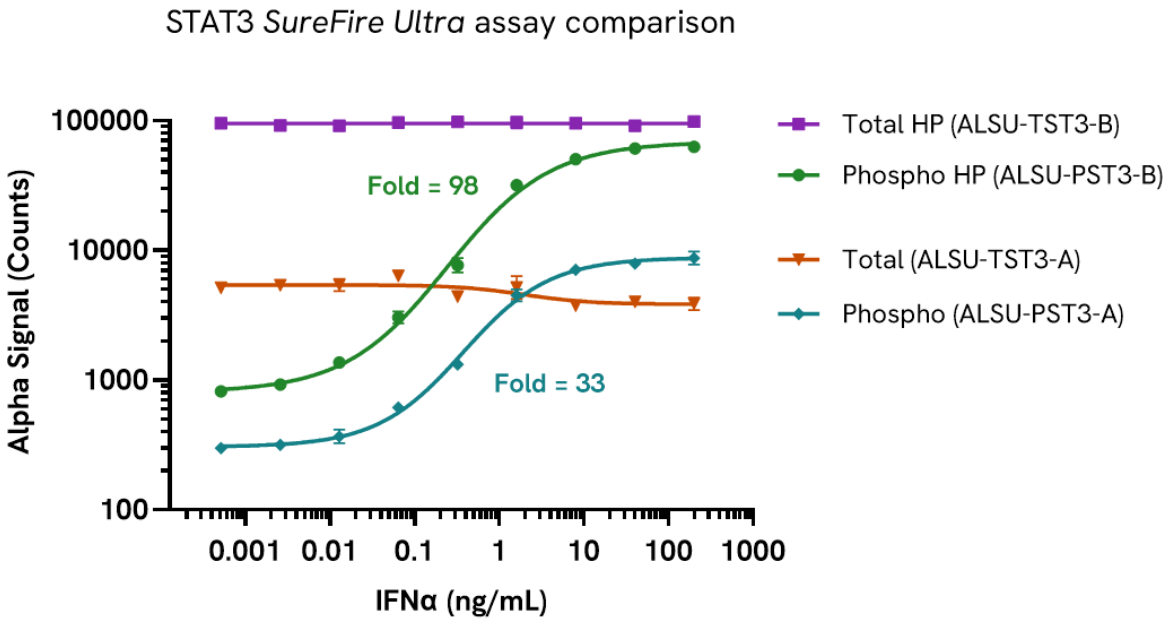
This assay kit contains antibodies which recognize the phospho-Tyr705 epitope and a distal epitope on STAT3. The protein detected by this kit corresponds to UniProt ID P40763. STAT3 is also known as Acute-phase response factor. These antibodies recognize STAT3 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

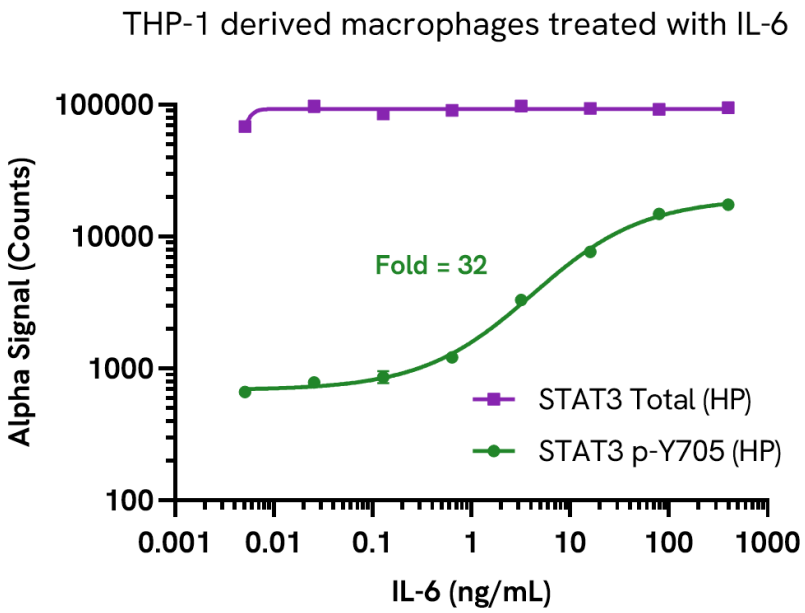
Positive Control Lysate: Prepared from A431 cells, cultured to confluence in T175 flasks in 10% FBS containing medium, then treated with 2 µg/mL EGF for 10 minutes and lysed with 5 mL of Lysis Buffer.

Representative data:

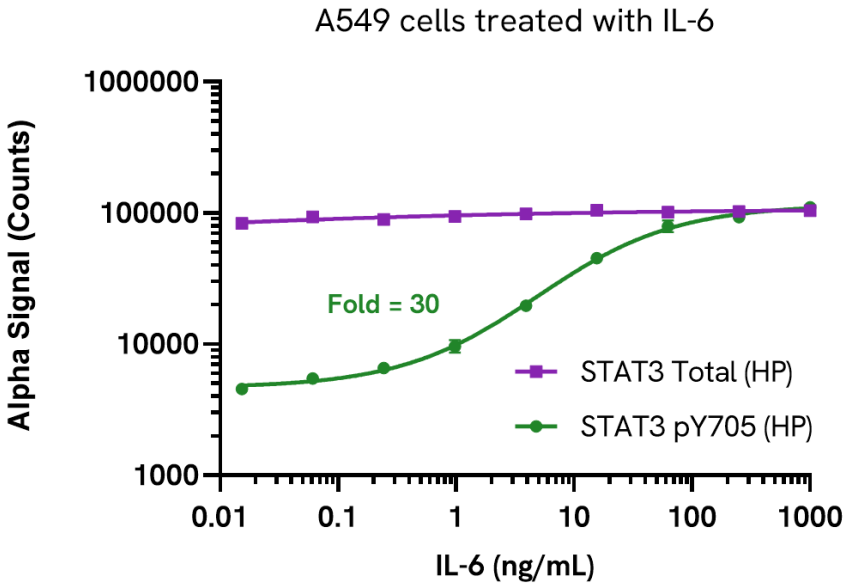
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded in a 96-well plate (50,000 cells/well) in complete medium containing 100 nM PMA and incubated for 24 hours. The THP-1 derived macrophages were starved in HBSS + 0.1% BSA for 2 hours and then stimulated with increasing concentrations of IFNα for 20 minutes. After treatment, the cells were lysed with Lysis Buffer and Phospho (Tyr705) and Total STAT3 levels were evaluated using respective *SureFire Ultra* kits. Equivalent to approximately 5,000 cells/datapoint.



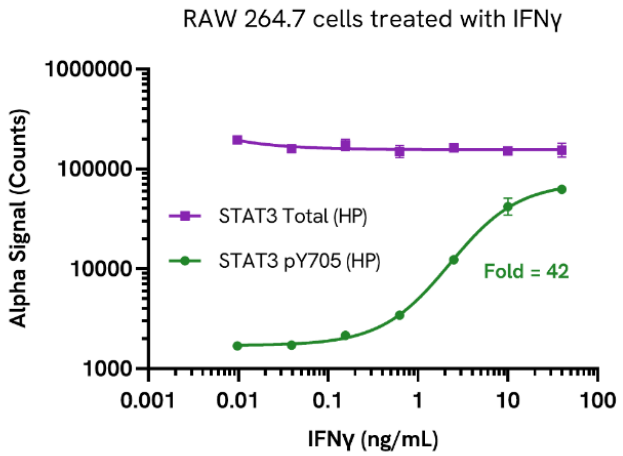
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded in a 96-well plate (50,000 cells/well) in complete medium containing 100 nM PMA and incubated for 24 hours. The THP-1 derived macrophages were starved in HBSS + 0.1% BSA for 2 hours and then stimulated with increasing concentrations of IL-6 for 20 minutes. After treatment, the cells were lysed with Lysis Buffer for 10 minutes and Phospho (Tyr705) and Total STAT3 levels were evaluated using respective *SureFire Ultra* High Performance kits. Equivalent to approximately 5,000 cells/datapoint.



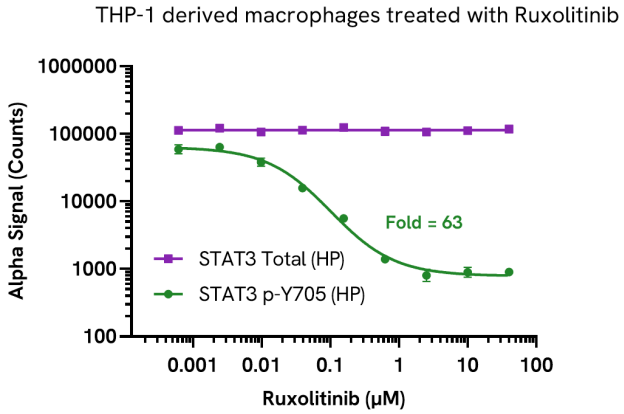
Data obtained with a 2-plate, 2-incubation protocol. A549 cells were seeded in a 96-well plate (20,000 cells/well) in complete medium and incubated overnight. Cells were starved for 20 hours and then stimulated with increasing concentrations of IL-6 for 30 minutes. After treatment, the cells were lysed with Lysis Buffer and Phospho (Tyr705) and Total STAT3 levels were evaluated using respective *SureFire Ultra* High Performance STAT3 kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol RAW264.7 cells were seeded in a 96-well plate (20,000 cells/well) in complete medium and incubated overnight. Cells were starved for 2 hours and then stimulated with increasing concentrations of IFNγ for 20 minutes. After treatment, the cells were lysed with Lysis Buffer and Phospho (Tyr705) and Total STAT3 levels were evaluated using respective *SureFire Ultra* High Performance kits. Equivalent to approximately 4,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded in a 96-well plate (50,000 cells/well) in complete medium containing 100 nM PMA and incubated for 24 hours. The THP-1 derived macrophages were treated with increasing concentrations of Ruxolitinib for 2 hours and then stimulated with 10 ng/mL IFNγ for 20 minutes. After treatment, the cells were lysed with Lysis Buffer for 10 minutes and Phospho (Tyr705) and Total STAT3 levels were evaluated using respective *SureFire Ultra* High Performance kits. Equivalent to approximately 5,000 cells/datapoint.



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