AlphaLISA™ *SureFire® Ultra™*

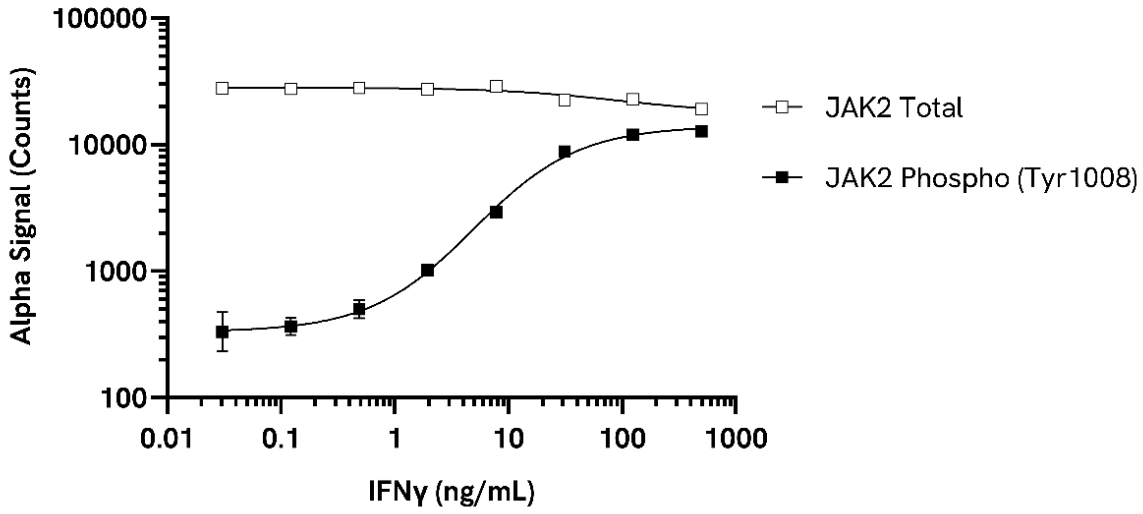
Human and Mouse p-JAK2 (Tyr1008) Detection Kit

Product number: ALSU-PJAK2-B500, ALSU-PJAK2-B10K, ALSU-PJAK2-B50K, ALSU-PJAK2-B-HV

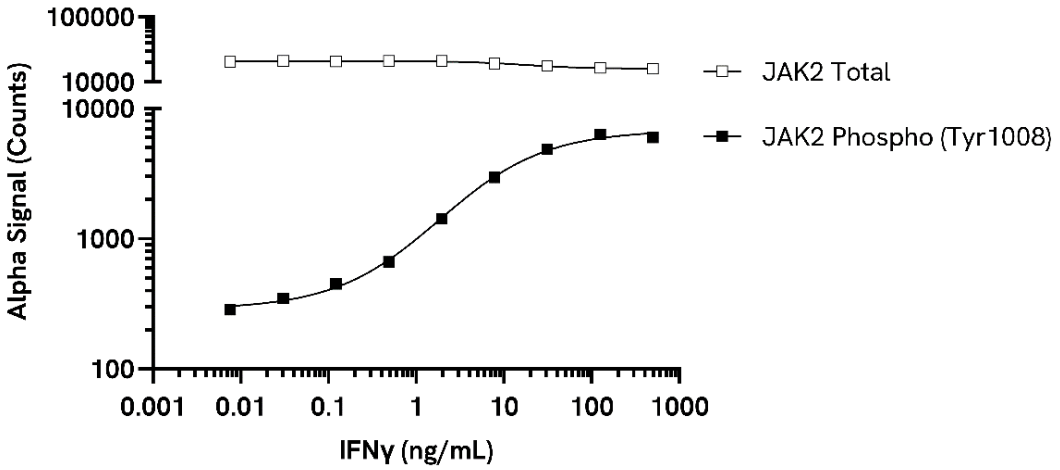
Kit specificity:
This assay kit contains antibodies which recognize the phospho-Tyr1008 epitope and a distal epitope on JAK2. The protein detected by this kit corresponds to UniProt ID O60674. JAK2 is also known as Janus kinase 2. These antibodies recognize JAK2 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:
Positive Control Lysate: Prepared from HEL92.1.7 cells seeded at 500K cells/mL and incubated overnight in 10% FBS containing medium. Cells were harvested, washed in HBSS + 0.1% BSA, adjusted to 4 x 10^6 cells/mL and treated with 0.5 mM Pervanadate for 15 minutes. Following treatment, cells were spun down and lysed with Lysis Buffer to a final concentration of 4 x 10^6 cells/mL.

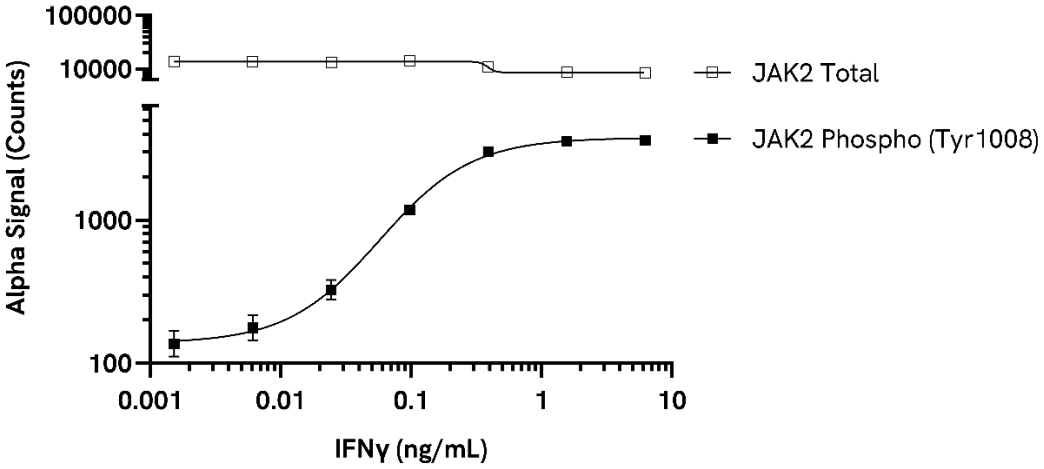
Representative data:
Data obtained with a 2-plate, 2-incubation protocol. PBMCs were isolated from healthy donors and cultured for 6 days in complete DMEM containing 20 ng/mL M-CSF to differentiate them into macrophages. Macrophages were seeded at 40K cells/well in a 96-well plate and incubated overnight. The cells were starved for 2 hours and then treated with IFNγ at the indicated concentrations for 20 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr1008) and Total JAK2 using respective *SureFire Ultra* kits. Equivalent to approximately 8,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 100K cells/well in a 96-well plate and incubated in complete medium containing 100 nM of PMA for 24 hours. Cells were starved with HBSS + 0.1% BSA for 2 hours, then treated with IFNγ at the indicated concentrations for 20 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr1008) and Total JAK2 using respective *SureFire Ultra* kits. Equivalent to approximately 16,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. RAW 264.7 cells were seeded at 40K cells/well in a 96-well plate and incubated overnight. Cells were starved for 2 hours and treated with IFNγ at the indicated concentrations for 20 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr1008) and Total JAK2 using respective *SureFire Ultra* kits. Equivalent to approximately 8,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. HEL92.1.7 cells were washed in HBSS + 0.1% BSA and seeded at 400K cells/well in a 96-well plate. Cells were treated with Ruxolitinib at the indicated concentrations for 15 minutes. Cells were spun down, lysed with Lysis Buffer and assayed separately for Phospho (Tyr1008) and Total JAK2 using respective *SureFire Ultra* kits. Equivalent to approximately 40,000 cells/datapoint.