AlphaLISA® *SureFire® Ultra™*

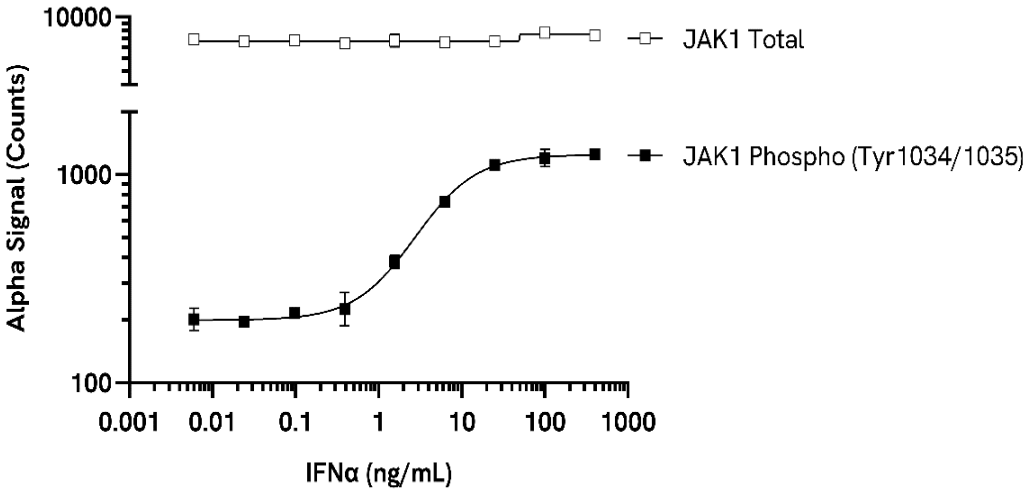
Human JAK1 Total Detection Kit

Product number: ALSU-TJAK1-A500, ALSU TJAK1-A10K, ALSU-TJAK1-A50K, ALSU-TJAK1-A-HV

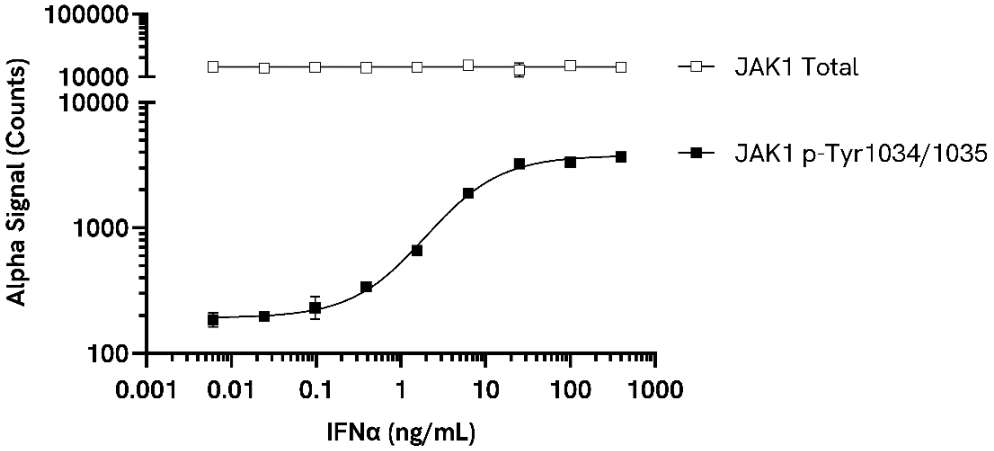
Kit specificity:
This assay kit contains antibodies which recognize distinct epitopes on JAK1. The protein detected by this kit corresponds to UniProt ID P23458. JAK1 is also known as Janus kinase 1. These antibodies recognize JAK1 of human origin. Other species should be tested on a case-by-case basis.

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 1 hour. Following treatment, cells were spun down and lysed with Lysis Buffer to a final concentration of 4 x 10^6 cells/mL.

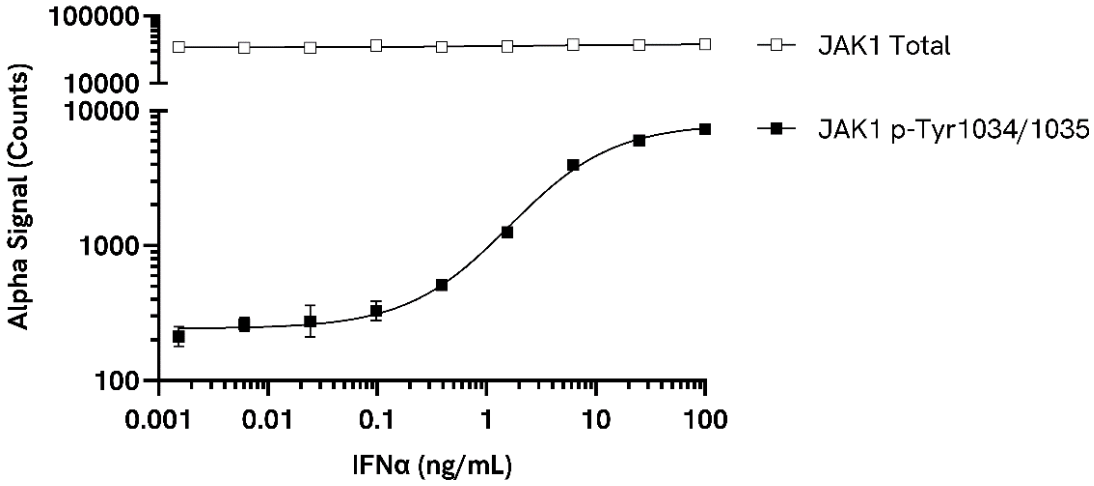
Representative data
Data obtained with a 2-plate, 2-incubation protocol. PBMCs were isolated from healthy donors and cultured for 6 days in complete DMEM containing 20 ng/mL M-CSF to differentiate them into macrophages. Macrophages were seeded at 30K cells/well in a 96-well plate and incubated overnight. The cells were treated with IFNα at the indicated concentrations for 10 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr1034/1035) and Total JAK1 using respective *SureFire Ultra* kits. Equivalent to approximately 6,000 cells/datapoint.



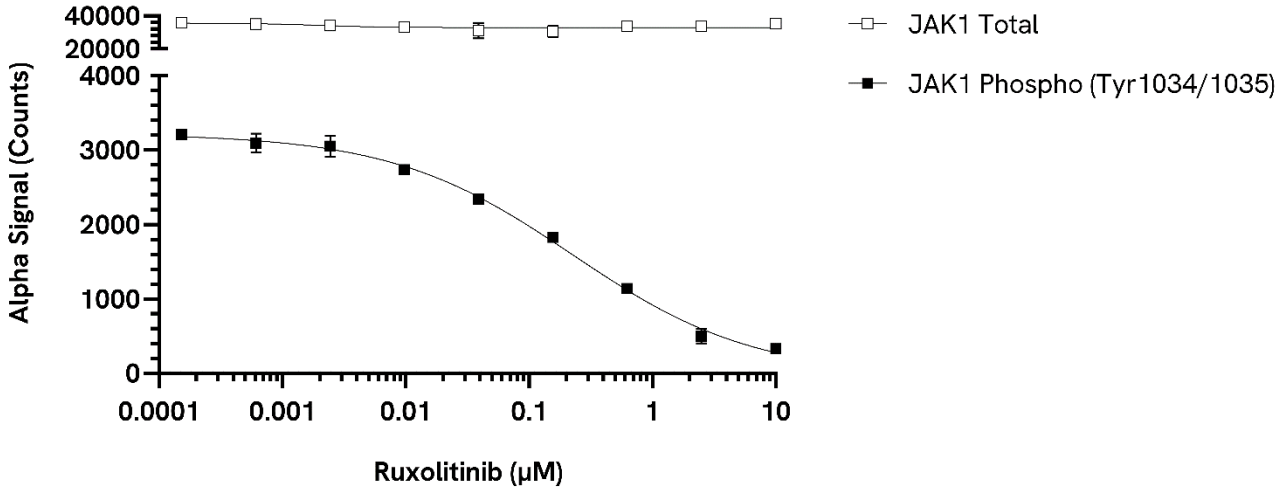
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 100K cells/well in a 96-well plate and incubated in complete medium containing 100 nM of PMA for 24 hours. Cells were then treated with IFNα at the indicated concentrations for 20 minutes in HBSS + 0.1% BSA. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Tyr1034/1035) and Total JAK1 using respective *SureFire Ultra* kits. Equivalent to approximately 20,000 cells/datapoint.



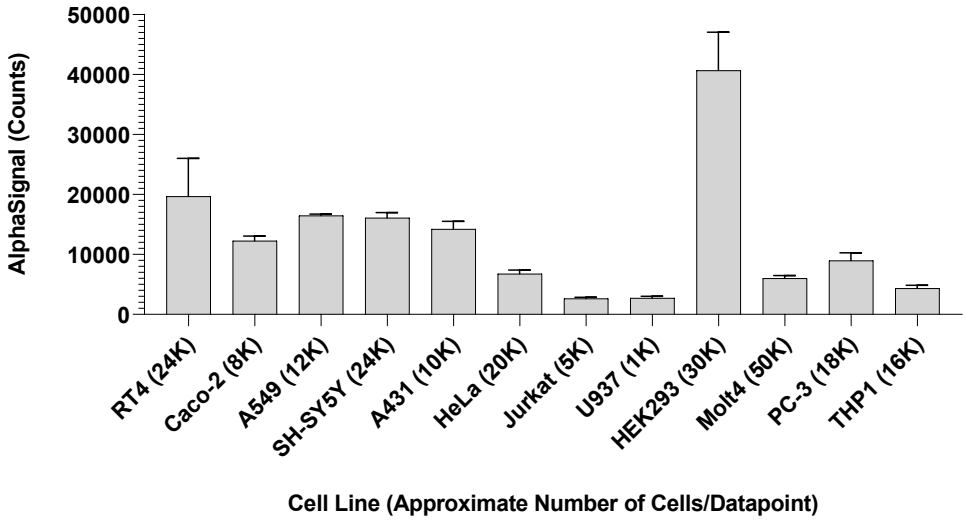
Data obtained with a 2-plate, 2-incubation protocol. HEL92.1.7 cells were harvested, washed in HBSS + 0.1% BSA and seeded at 400K cells/well in a 96-well plate. Cells were treated with IFNα at the indicated concentrations for 10 minutes. Cells were spun down, lysed with Lysis Buffer and assayed separately for Phospho (Tyr1034/1035) and Total JAK1 using respective *SureFire Ultra* kits. Equivalent to approximately 40,000 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 stimulated with 100 ng/mL IFNα for 10 minutes and then treated with Ruxolitinib at the indicated concentrations for 15 minutes. Cells were spun down, lysed with Lysis Buffer and assayed separately for Phospho (Tyr1034/1035) and Total JAK1 using respective *SureFire Ultra* kits. Equivalent to approximately 40,000 cells/datapoint.



Data obtained from measurement of JAK1 Total in various cell types lysed with Lysis Buffer. Approximate number of cells/datapoint is indicated for the various cell lines.



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