

Research use only. Not for use in diagnostic procedures.

## AlphaLISA<sup>®</sup> SureFire<sup>®</sup> Ultra<sup>™</sup>

# Human and Mouse JAK2 Total Detection Kit

Product number:

ALSU-TJAK2-A500, ALSU-TJAK2-A10K,

ALSU-TJAK2-A50K, ALSU-TJAK2-A-HV



### Kit specificity:

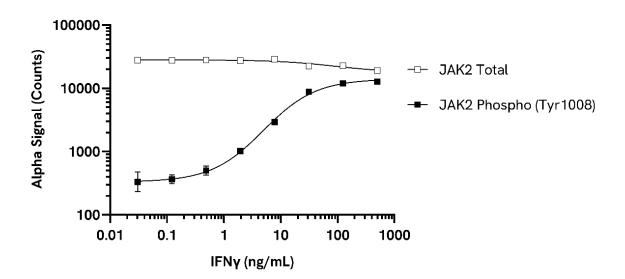
This assay kit contains antibodies that recognize distinct epitopes on JAK2. The protein detected by this kit corresponds to UniProt ID 060674. JAK2 is also known as Janus Kinase 2. These antibodies recognize JAK2 of human and mouse origin. Other species should be tested on a case-by-case basis.

### Control lysate information:

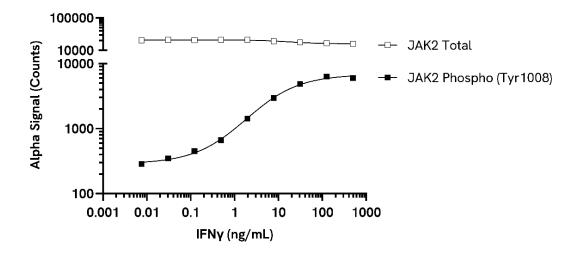
Positive Control Lysate: Prepared from HEL92.1.7 cells seeded at 500K cells/mL and incubated overnight in 10% FBS containing medium. Cells were harvested, washed in HBSS + 0.1% BSA, adjusted to 4 x 10<sup>6</sup> cells/mL and treated with 0.5 mM Pervanadate for 15 minutes. Following treatment, cells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

#### **Representative data**

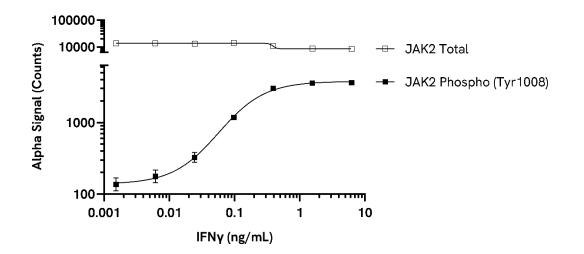
Data obtained with a 2-plate, 2-incubation protocol. PBMCs were isolated from healthy donors and cultured for 6 days in complete DMEM containing 20 ng/mL M-CSF to differentiate them into macrophages. Macrophages were seeded at 40K cells/well in a 96-well plate and incubated overnight. The cells were starved for 2 hours and then treated with IFNy 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.



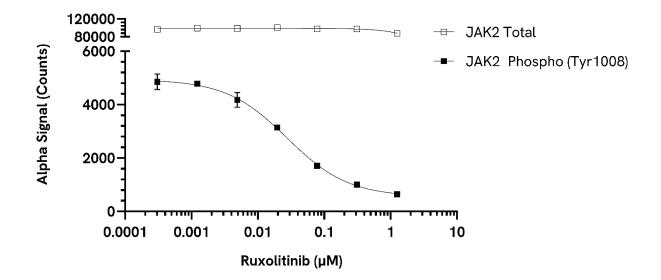
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 100K cells/well in a 96-well plate and incubated in complete medium containing 100 nM of PMA for 24 hours. Cells were starved with HBSS + 0.1% BSA for 2 hours, then treated with IFN $\gamma$  at the indicated concentrations 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.



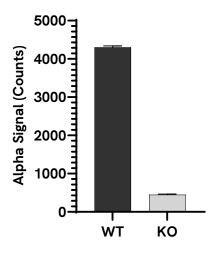
Data obtained with a 2-plate, 2-incubation protocol. RAW 264.7 cells were seeded at 40K cells/well in a 96-well plate and incubated overnight. Cells were starved for 2 hours and treated with IFN<sub>Y</sub> at the indicated concentrations for 20 minutes. Cells were lysed with Lysis Buffer and assayed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



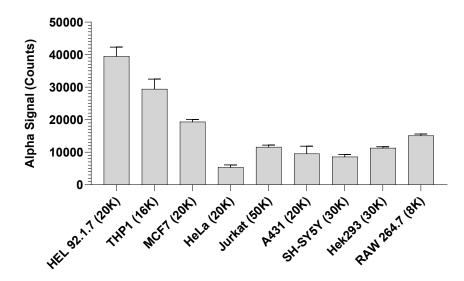
Data obtained with a 2-plate, 2-incubation protocol. HEL92.1.7 cells were washed in HBSS + 0.1% BSA and seeded at 400K cells/well in a 96-well plate and Incubated overnight. Cells were treated with Ruxolitinib at the indicated concentrations for 15 minutes. Cells were spun down, lysed with Lysis Buffer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



A549 wild type (WT) and A549-JAK2 knockout (KO, Abcam ab267113) cell lines were seeded at 40K cells/well in a 96 well plate. Cells were lysed in Lysis Buffer then assayed separately for Total JAK2. Equivalent to approximately 4,000 cells/datapoint.



Data obtained from measurement of JAK2 Total in various cell types lysed with Lysis Buffer. Approximate number of cells/datapoint is indicated for the various cell lines.



Cell Line (Approximate Number of Cells/Datapoint)

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