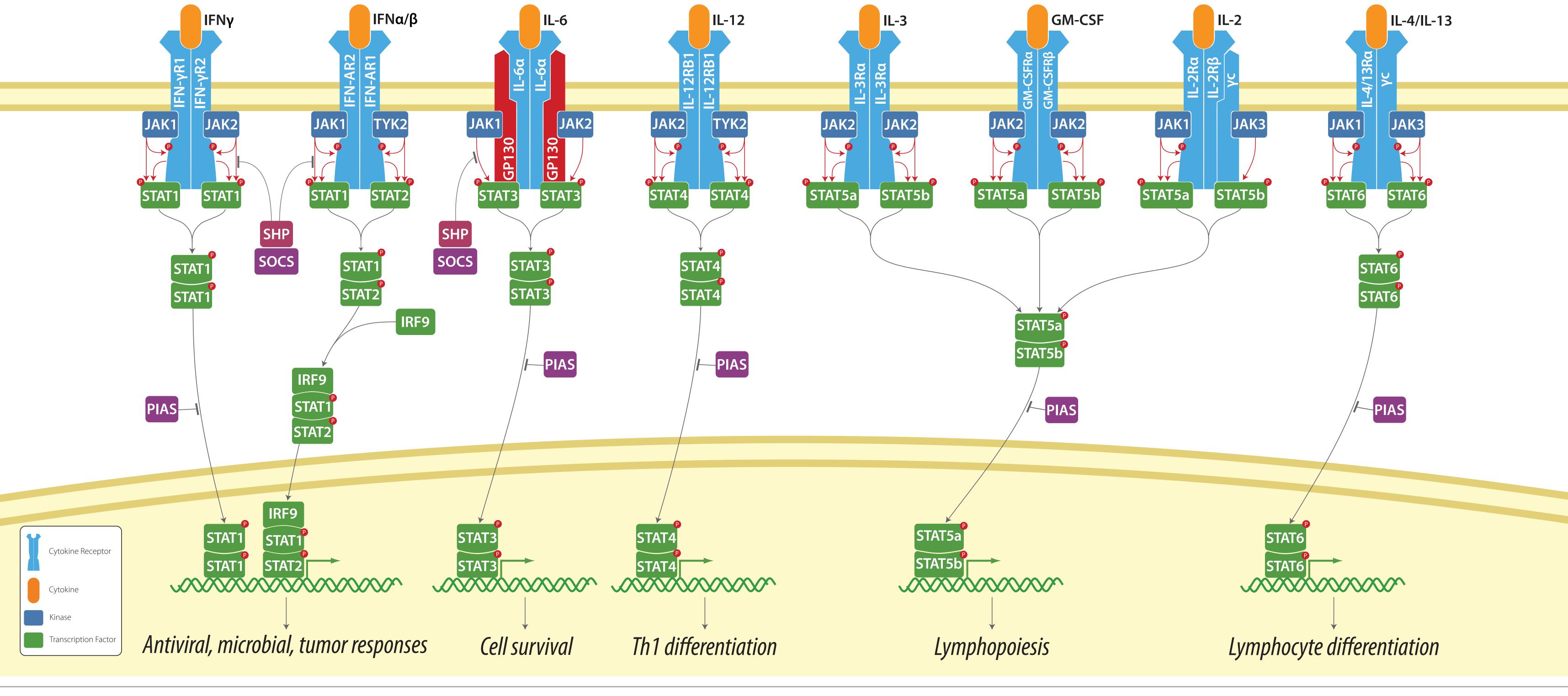
Jak/Stat signaling pathway



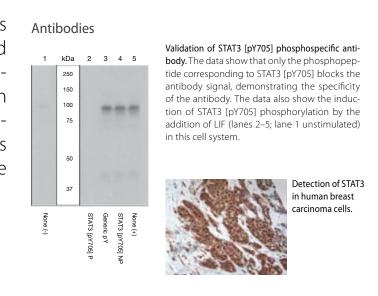
Your path to performance

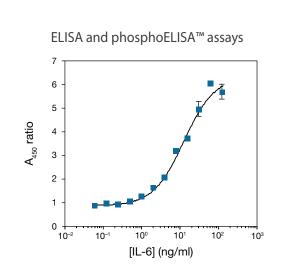
Empower your research using Invitrogen's comprehensive portfolio of products and services to investigate the Jak/Stat pathway—high-quality reagents for basic research and assay development, validated biochemical and cell-based assays, and world-class profiling and screening services. Learn more at www.invitrogen.com/jakstat.

Antibodies

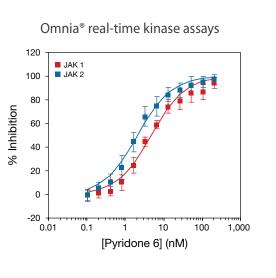
Antibodies

Antibodies

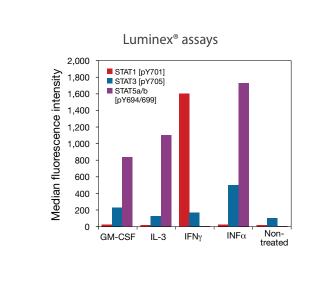




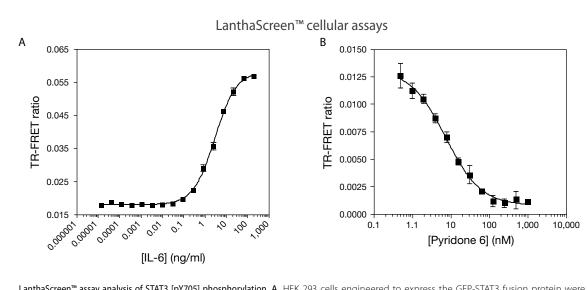
Cellular phosphoanalysis of endogenously expressed substrates such as STAT3 achieved using the phosphoELISA™ kits. ME180 SIE-bla cells were seeded in a 96-well assay plate and allowed to adhere overnight. The cells were stimulated with serial dilutions of IL-6 for 30 min before the medium was removed and lysis buffer was added. Cell lysates (100 µl/well) were then processed using the STAT3 [pY705] ELISA kit according to the supplied protocol, and the A_{sco} was determined.



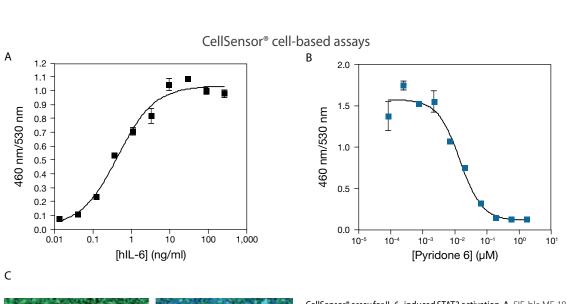
Omnia® assay analysis of JAK kinase activity. Omnia® real-time kinase assays were performed in a 50 µl reaction volume with either 10 µM Omnia® Plus Tyr 12 peptide substrate (JAK1) or 2 µM HOP83 peptide substrate (JAK2) and ATP at the K_{mapp} concentration for JAK2. Data were collected in real time for 1 hr and then the reaction curves were fit to a linear reaction rate at each individual inhibitor concentration. The reactions were performed with enzyme concentrations equal to the EC $_{30}$ values.

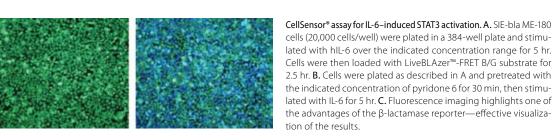


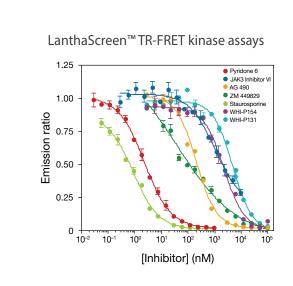
STAT 1, 3, and 5 phosphorylation measured in one sample using the Luminex® bead-based platform for multiplexing capabilities. TF-1 cells were serum starved overnight and then stimulated with the specific cytokines for 15 min. Unstimulated cells were used as a no-treatment control. Expected levels of STAT proteins were measured following specific treatments using the STAT 1, 3, 5 Plex Antibody Bead Kit.



LanthaScreen™ assay analysis of STAT3 [pY705] phosphorylation. A. HEK 293 cells engineered to express the GFP-STAT3 fusion protein were added to a 384-well assay plate and allowed to adhere overnight. Cells were then stimulated with serial dilutions of IL-6 for 30 min before the medium was removed and lysis buffer was added. The TR-FRET signal of the cell lysates was determined following a 20 min incubation with Tb-labeled anti–[pY705] STAT3 antibody. B. The assay was performed similar to as described in A, except serial dilutions of the inhibitor were allowed to incubate with the cells for 90 min before stimulation with an EC₈₀ concentration of IL-6. After lysis, 20 µl of lysate was transferred to a white, low-volume assay plate before addition of the Tb-labeled antibody.







LanthaScreen[™] assay analysis of JAK2 kinase activity. LanthaScreen[™] JAK2 kinase assays were performed in a 10 μ l reaction volume with 1% DMSO, 400 nM fluoresceinpoly GT, and ATP at the K_{mapp} concentration using a 1 hr incubation. Detection was achieved by adding 10 μ l of antibody/EDTA mix to a final concentration of 2 nM antibody and 10 mM EDTA. TR-FRET measurements were obtained, then inhibitor studies were performed using enzyme concentrations equal to the EC₈₀ values.

