

STAT3 Reporter Assay Service

Boster Bio custom STAT3 reporter assay utilizes the proprietary STAT3-RE (Response Element) reporter HEK 293 cell line system. Signal Transducers and Activators of Transcription-3 (STAT3) plays an important role in cellular processes, of which dysregulation is associated with tumor progression of various cancers. STAT3 reporter system is designed to monitor the transcriptional activity of STAT3. STAT3 reporter assay services include screening of agonists/antagonists that affect the induction of phospho-STAT3 or STAT3 transcriptional activity, EC₅₀/IC₅₀ evaluation of agonists/antagonists/inhibitory antibodies, and functional analysis of STAT3 signaling pathways. STAT3 reporter system is also suitable for screening of inhibitors/antagonists against IL-6 or IL-6 receptor. Assay results will contain a written report and downloadable Excel- or Prism-based data.

Figure 1. Analysis of the IL-6-mediated STAT3 induction activity in stable STAT3-RE Reporter HEK 293 cells. The STAT3-RE Reporter HEK 293 cell line was plated in 24-well plates at 0.2×10^6 cells/well. After 16 h, cells were stimulated with 10 and 50 ng/ml IL-6 for 6 h and 16 h. Luciferase activity was then analyzed using the luciferase reporter assay reagent.

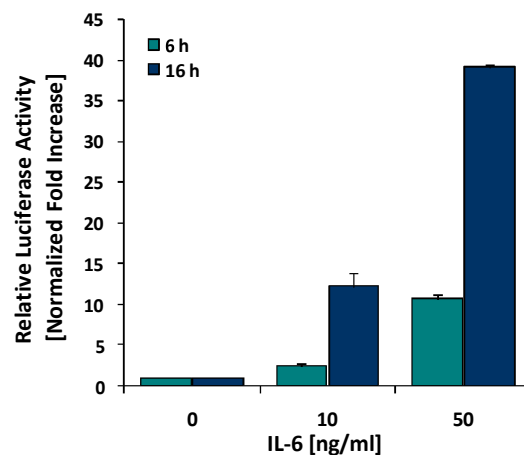


Figure 2. Inhibitory effects of piceatannol and curcumin on IL-6-mediated STAT3 activation in stable STAT3-RE Reporter HEK 293 cells. The STAT3-RE Reporter HEK 293 cell line was plated in 96-well white plates at 5×10^4 cells/well. After 16 h, cells were pretreated with 5, 10 and 50 μ M piceatannol or curcumin for 1 h. Cells were then stimulated with 10 ng/ml IL-6 for 16 h. Luciferase activity was then analyzed by directly adding the complete mixture of luciferase reporter assay reagent into each well of the plate. After 5 min, the plate was read in a plate luminometer. *Note: Piceatannol and curcumin are known inhibitors of STAT3 phosphorylation.*

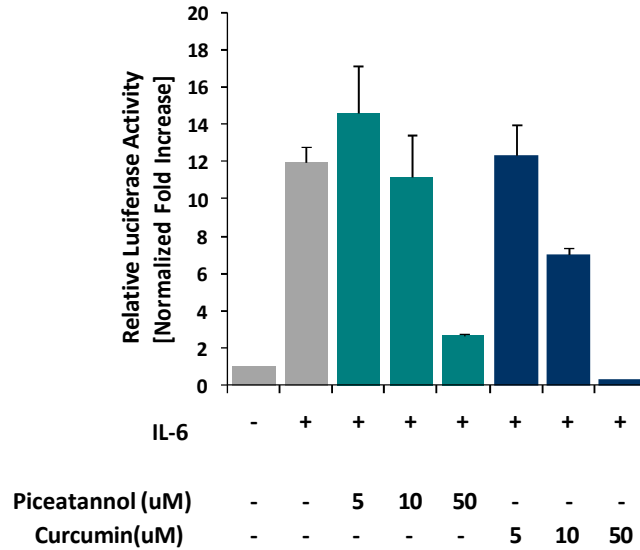


Figure 3. Enhancive effects of pervanadate on IL-6-mediated STAT3 activation in stable STAT3-RE Reporter HEK 293 cells. The STAT3-RE Reporter HEK 293 cell line was plated in 96-well white plates at 5×10^4 cells/well. After 16 h, cells were pretreated with 5, 10 and 50 μ M pervanadate for 1 h. Cells were then stimulated with 10 ng/ml IL-6 or vehicle for 16 h. Luciferase activity was then analyzed by directly adding the complete mixture of luciferase reporter assay reagent into each well of the plate. After 5 min, the plate was read in a plate luminometer. *Note: Pervanadate is a known inhibitor of tyrosine phosphatase. Treatment of pervanadate results in enhancement of tyrosine phosphorylation of STAT3.*

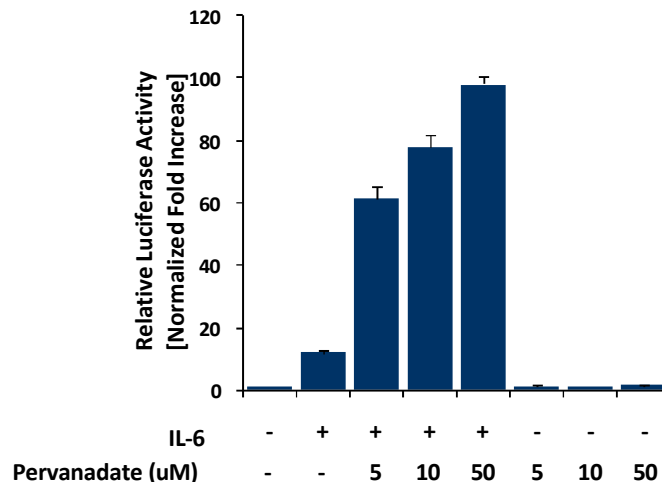
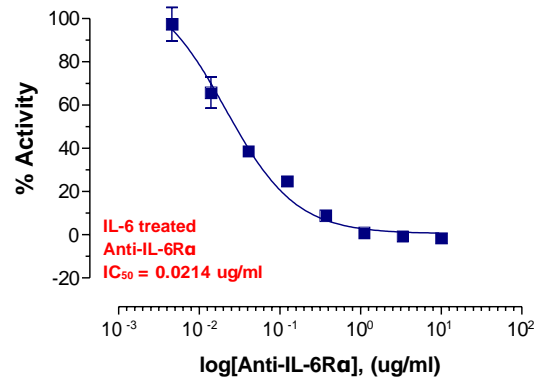
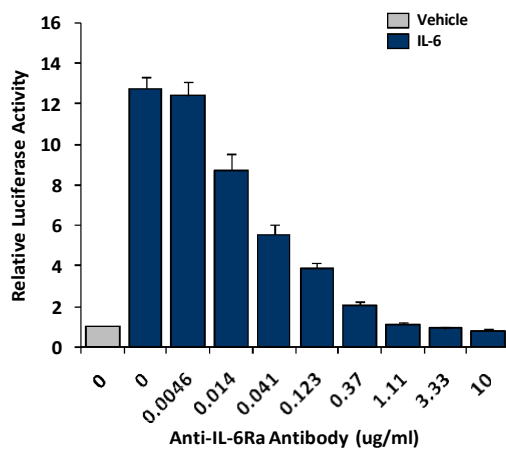


Figure 4. Inhibitory effects of human IL-6Ra antibody (MAB227, R & D Systems) on IL-6-mediated IL-6R activation in stable STAT3-RE Reporter HEK 293 cells. The STAT3-RE Reporter HEK 293 cell line was plated in 96-well white plates at 5×10^4 cells/well. After 16 h, cells were pretreated with various concentrations of anti-hIL-6Ra antibody for 30 min. Cells were then stimulated with 10 ng/ml IL-6 (**A**) or PMA (**B**) for 16 h. Luciferase activity was then analyzed by directly adding the complete mixture of luciferase reporter assay reagent into each well of the plate. After 5 min, the plate was read in a plate luminometer. *Note: The human IL-6Ra antibody (monoclonal mIgG1 clone #17506; Cat. No. MAB227) is a known neutralizing antibody from R & D Systems. The antibody specifically inhibited the IL-6 mediated (but not PMA mediated) STAT3/Luciferase induction. The IC_{50} of 0.0214 $\mu\text{g/ml}$ is the concentration of the antibody that produces half maximal inhibition of IL-6Ra activity.*

A.



B.

