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Data Sheet

STAT3 Reporter (Luc) - HEK293 Cell line (Puromycin)

Catalog #: 79800-P

Product Description
The STAT3 Reporter (Luc)-HEK293 cell line is designed for monitoring STAT3 signal transduction pathway. It contains a firefly luciferase gene driven by STAT3 response elements located upstream of the minimal TATA promoter. After activation by cytokines and growth factors, endogenous STAT3 binds to the DNA response elements, inducing transcription of the luciferase reporter gene.

Format
Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO.

Applications
- Monitor the STAT3 signaling pathway activity
- Screen for activators or inhibitors of the STAT3 signaling pathway

General Culture Conditions

Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 1N (BPS Bioscience #79801): Thaw Medium 1 (BPS Bioscience #60187) and 0.5 µg/ml of Puromycin (InvivoGen, #ant-pr-1).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1N.

Storage
Immediately upon receipt, store in liquid nitrogen.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, and transfer to a tube containing 10 ml of Thaw Medium 1 **(no puromycin)**. Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 **(no puromycin)**, transfer resuspended cells to a T25 flask and culture in 37°C CO_2 incubator. At first passage switch to Growth Medium 1N **(contains puromycin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1N and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:5 to 1:10 weekly or twice a week.

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Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with a higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1N and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Mycoplasma testing
The cell line has been screened using the PCR-based Venor™GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

Assay performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- Human IL-6 (R&D Systems #206-IL)
- JAK inhibitor CP690550 (Cayman #11598)
- Anti-IL-6R antibody (R&D Systems #MAB227)
- Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)
- Growth Medium 1N (BPS Bioscience #79801)
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- One-Step luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

A. Human IL-6 dose response

1. Harvest STAT3 reporter (Luc)-HEK293 cells and seed cells at a density of 30,000-40,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.
2. Add threefold serial dilution of IL-6 in 10 µl of assay medium to IL-6-stimulated wells.

   a. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of STAT3 reporter activity).

   b. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

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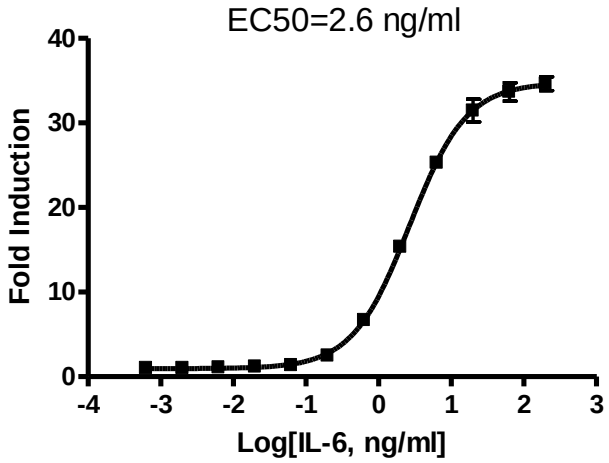
3. Incubate at 37°C with 5% CO_2 for 5-18 hours.
4. Prepare ONE-Step™ Luciferase Assay reagent as directed and add 100 µl per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all measurements.

Figure 1. IL-6 Dose Response in STAT3 (Luc) Reporter HEK293 Cells. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without IL-6 treatment.

The EC_{50} of IL-6 in this cell line is ~2 ng/ml.



B. Inhibition of IL-6 induced STAT3 activity by JAK inhibitor

1. Harvest STAT3 reporter (Luc)-HEK293 cells and seed cells at a density of 30,000-40,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Next day, treat cells with three-fold serial dilution of CP690550 (JAK inhibitor; Cayman, #11598) in 90 µl of assay medium. Incubate cells at 37°C with 5% CO_2 for 1-2 hours. For control cells without CP690550, change to 90 µl of assay medium with no treatment.

3. Set up each treatment in at least triplicates:

   a. Add 10 µl of diluted human IL-6 in assay medium to stimulated wells (final IL-6 concentration = 10 ng/ml).

   b. Add 10 µl of assay medium to the unstimulated control wells (for determining the basal activity).

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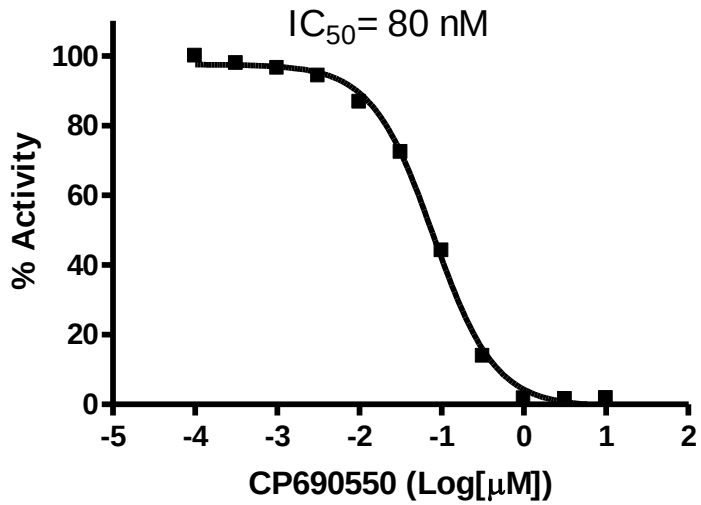
c. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

4. Incubate at 37°C with 5% CO_2 for 5-18 hours.

5. Prepare ONE-Step™ Luciferase Assay reagent as directed and add 100 µl per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all measurements.

6. Data Analysis: Obtain background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 2. Inhibition of IL-6-induced Reporter Activity by JAK Inhibitor CP690550 in STAT3 (Luc) Reporter HEK293 Cells. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IL-6 in the absence of CP690550 was set at 100%**.**



C. Inhibition of IL-6 induced STAT3 activity by anti-IL-6R antibody

1. Harvest STAT3 reporter (Luc)-HEK293 cells and seed cells at a density of 30,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Next day, treat cells with three-fold serial dilution of anti-IL-6R antibody (R&D Systems, #MAB227) in 90 µl of assay medium for one hour. For control cells without antibody treatment, change to 90 µl of fresh assay medium.

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3. Set up each treatment in at least triplicates:

   a. Add 10 µl of diluted human IL-6 in assay medium to stimulated wells (final IL-6 concentration = 10 ng/ml).

   b. Add 10 µl of assay medium to the unstimulated control wells (for determining the basal activity).

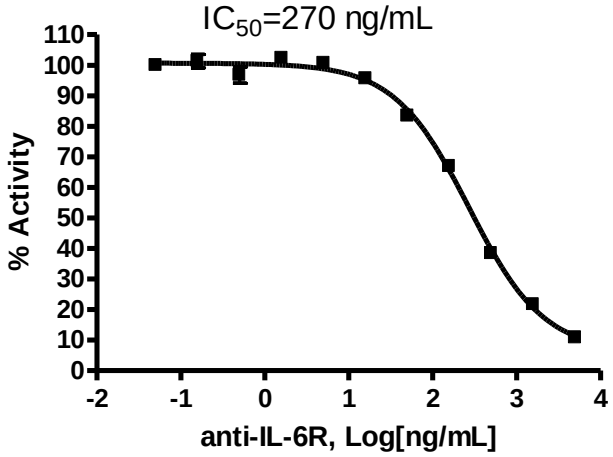
   c. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

4. Incubate at 37°C with 5% CO_2 for 18-24 hours.

5. Prepare ONE-Step™ Luciferase Assay reagent as directed and add 100 µl per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all measurements.

6. Data Analysis: Obtain background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 3. Inhibition of IL-6-induced Reporter Activity by Anti-IL-6R Antibody in STAT3 (Luc) Reporter HEK293 Cells. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IL-6 in the absence of anti-IL-6R antibody was set at 100%**.**



References

1. Tian S., *et al., Blood*. 1994; **84(6)**:1760-1764.
2. Zhong, Z., *et al., Science*. 1994; **264(5155):**95-98.

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Related Products

Product	Cat. #	Size
Thaw Medium 1	60187	100 ml
Growth Medium 1N	79801	500 ml
STAT3 Reporter Kit	79730	500 rxns.
STAT3 Luciferase Reporter Lentivirus	79744	2 x 500 µl
Human IL-6	90196-B	20 µg
ONE-Step Luciferase Detection Reagent	60690-1	10 ml
STAT3, GST-tag	75003	20 µg
STAT5 Reporter (Luc) – Ba/F3 Cell line	79722	2 vials