



STAT3 Luciferase Reporter- HEK293 Cell Line

Catalog number: RC1014

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

STAT3 Luciferase Reporter-HEK293 Cell Line

Catalog Number: RC1014, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)

Contents: Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.

Description: The STAT3 Luciferase Reporter cell line is a stably transfected HEK 293 cell line which expresses Renilla luciferase reporter under the transcriptional control of the STAT3 responsive promoter, so that the cell line is designed to measure the transcriptional activity of STAT3. As a transcription factor, Signal Transducer and Activator of Transcription 3 (STAT3) is activated through phosphorylation at tyrosine 705 in response to various cytokines including IL-6, interferons, epidermal growth factor, hepatocyte growth factor and leukemia inhibitory factor. The phosphorylated STAT3 forms homodimers or heterodimers with STAT1, and the dimers translocate to nucleus in which DNA binding/promoter induction occurs. The STAT3 induction by IL-6 is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of STAT3

Application Details:

Application:

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

Culture conditions:

Cells should be grown at 37°C with 5% CO₂ using DMEM medium supplemented with 10% FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin. It is recommended to quickly thaw the frozen cells upon receipt or from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Puromycin, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin, transfer resuspended cells to T25 flask and culture in 37°C-CO₂ incubator. Leave the T25 flask in the incubator for 2~4 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are over 90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence. To passage the cells, detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cells suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly.

Functional validation:

A. Response of STAT3 HEK293 cells to IL-6 (Figure 1). 1. Harvest STAT3 HEK293 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5×10^4 cells/well. 2. Incubate cells at 37°C in a CO₂ incubator for overnight. 3. The next day, stimulate cells with different concentrations of IL-6. 4. Incubate at 37°C in a CO₂ incubator for 6-16 hours. 5. Add 50 µl of luciferase assay reagent per well. 6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer. B. Inhibition of IL-6-induced STAT3 activity (Figure 2). 1. Harvest STAT3 HEK293 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5×10^4 cells/well. 2. Incubate cells at 37°C in a CO₂ incubator for overnight. 3. The next day, pre-treat cells with different concentrations of anti-IL-6Ra antibody (MAB227, R & D Systems) for 1 hour. 4. Add 10 ng/ml IL-6 and incubate cells at 37°C in a CO₂ incubator for 6-16 hours. 5. Add 50 µl of luciferase assay reagent per well. 6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

STAT3 Luciferase Reporter-HEK293 Cell Line (RC1014) Images

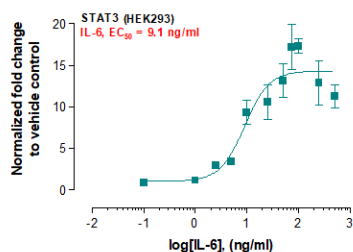


Fig-1: Induction of STAT3 activity by IL-6 in STAT3 HEK293 cells.

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