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Data Sheet

STAT5 Reporter (Luc) – Ba/F3 Cell line

Catalog #: 79772

Product Description
The STAT5 Reporter (Luc)-Ba/F3 cell line is designed for monitoring STAT5 signal transduction pathways. It contains a firefly luciferase gene driven by the STAT5 response element located upstream of the minimal TATA promoter. After activation by cytokines or growth factors, endogenous STAT5 binds to the DNA response elements, inducing transcription of the luciferase reporter gene.

Application
- Monitor STAT5 signaling pathway activity
- Screen for activators or inhibitors of STAT5 signaling pathway

Format
Each vial contains ~3 x 10^6 cells in 1 ml of FBS with 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Host Cell
Ba/F3: IL-3 dependent murine pro B cell line. Non-adherent cells.

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.

General Culture Conditions
Thaw Medium 8 (BPS Bioscience, #79652) plus 5 ng/mL mouse IL-3: RPMI1640 medium (Life Technologies, #A10491-01) supplemented with 10% heat-inactivated FBS (Life Technologies, #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01), 5 ng/ml mIL-3 (R&D systems, #403-ML-010).

Growth Medium 8A (BPS Bioscience, #79653) plus 5 ng/mL mouse IL-3: Thaw Medium 8 (BPS Bioscience, #79652), 1 µg/ml of Puromycin (Takara, #631306), 5 ng/ml mIL-3 (R&D systems, #403-ML-010).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 8A with the addition of 5 ng/ml mIL-3. ***Note***: mIL-3 is essential for Ba/F3 cell maintenance. Thaw Medium 8 and Growth Medium 8A do not contain mIL-3.

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It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 8 plus 5 ng/ml mIL-3 **(no Puromycin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 8 plus 5 ng/ml mIL-3 **(no Puromycin)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 1 – 2 ml of Thaw Medium 8 plus 5 ng/ml mIL-3 **(no Puromycin)**. At first passage, switch to Growth Medium 8A **(contains Puromycin)** plus 5 ng/ml IL-3. Cells should be split before they reach 2.0 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 x 10^6 cells/ml. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml.

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

Materials Required but Not Supplied
- mIL-3 (R&D systems, #403-ML-010)
- Assay Medium: Thaw Medium 8 (BPS Bioscience, #79652)
- Growth Medium 8A (BPS Bioscience, #79653)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

mIL-3 Dose Response
1. Harvest STAT5 Reporter (Luc) Ba/F3 cells from culture in Growth Medium 8A containing 5 ng/ml mIL-3. Wash cells once with assay medium (containing no mIL-3); resuspend the cells in assay medium and seed cells into a white opaque 96-well microplate at a density of ~20,000 cells per well in 90 µl of assay medium. Leave a couple wells empty for use as a background control.

2. Prepare threefold serial dilution of mIL-3 in assay medium. Add 10 µl of diluted mIL-3 to mIL-3 stimulated wells.

3. Add 10 µl of assay medium to the unstimulated control wells (for measuring the uninduced level of STAT5 reporter activity).

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

5. Incubate at 37°C with 5% CO_2 for 5-16 hours.

6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30

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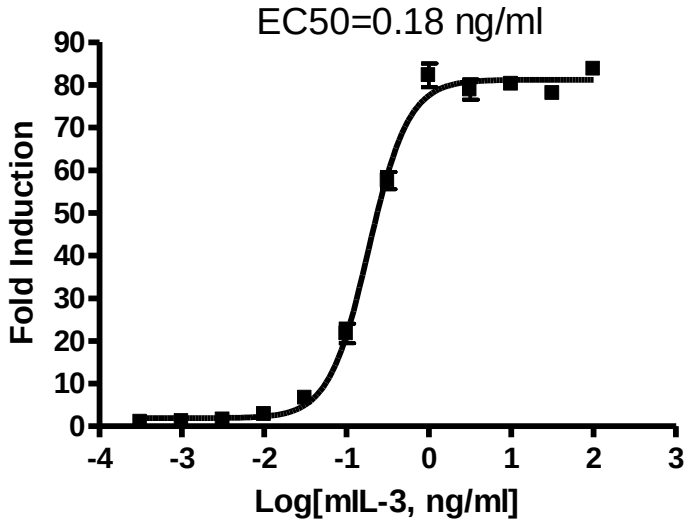
minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

Figure 1. mIL-3 dose response in STAT5 reporter (Luc)-Ba/F3 cells. Cells were treated with mIL-3 for ~ 16 hours. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without mIL-3 treatment.

The EC50 of mIL-3 in this cell line is ~0.18 ng/ml.



Related Products

Product	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 8	79652	100 ml
Growth Medium 8A	79653	500 ml
Mouse IL-3	403-ML-010	2 µg
Mouse IL-3	403-ML-010	10 µg

References

1. Palacios, R., Henson, G., Steinmetz, M., McKearn, J. P. (1984). Interleukin-3 supports growth of mouse pre-B-cell clones in vitro. *Nature*, **309 (5964)**: 126-131.
2. Jaster R, Tschirch E, Bittorf T, Brock J. (1999). Role of STAT5 in interferon-alpha signal transduction in Ba/F3 cells. *Cell Signal.*, **11(5)**:331-5.

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