9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

14-147ACL: STAT3 Leeporter™ Luciferase Reporter-Ba/F3 Cell Line

Application : Functional Assay

Description

The STAT3 Leeporter™ Luciferase Reporter cell line is a stably transfected Ba/F3 cell line which expresses Renilla luciferase reporter gene 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. The STAT3 induction by interferon gamma is shown in Figure 1.

Product Info

Amount :	1 Vial
Content :	Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Storage condition :	Immediately upon receipt, store in liquid nitrogen.

Application Note

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% CO_2 using RPMI medium supplemented with 10% heat-inactivated FBS, 1 mM sodium pyruvate, 10 mM HEPES and 1% Pen/Strep, plus 5 ng/ml mIL-3 (Note: mIL-3 is essential for Ba/F3 cell maintenance), plus 3 μg/ml of Puromycin. (Note: The parental Ba/F3-Puro cell line (Part #14135CCL) is a blank vector-transfected stable cell line which also requires 3 μg/ml of Puromycin for cell culture maintenance! Puromycin can be omitted during the reporter cell assays).

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-CO_2 incubator.

Monitor the cell viability by counting cells daily for 1~3 days until cells completely recover viability as cells are doubling daily. Once cells are over 90% confluent, harvest cells by centrifugation and passage cells. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence.

To passage the cells, transfer cells to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.

9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

Functional validation:

A. Response of STAT3 Leeporter™ – Ba/F3 cells to mIFN-gamma.

1. Harvest STAT3 Leeporter™ – Ba/F3 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium without IL-3 at 1 x 10^5 cells/well.

2. Right after plating cells, stimulate cells with various concentrations of mouse IFN-gamma and incubate cells at 37°C in a CO_2 incubator for 16 hours.

3. Equilibrate the plate to room temperature for 10 minutes.

4. Add 50 µl of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.

5. Read the plate in 1-5 minutes to measure luminescence using a microplate luminometer.

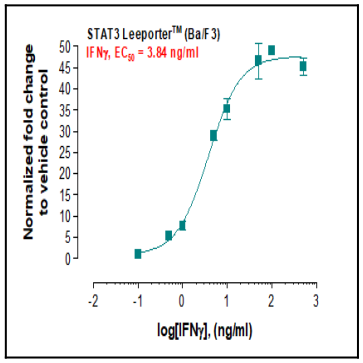

Fig-1: Induction of STAT3 activity by mIFN-gamma in STAT3 Leeporter™ – Ba/F3 cells.