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# 14-135ACL: STAT5 Leeporter™ Luciferase Reporter-Ba/F3 Cell Line

**Application :** Functional Assay

## Description

The STAT5 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 STAT5 responsive promoter, so that the cell line is designed to measure the transcriptional activity of STAT5. As a transcription factor, Signal Transducer and Activator of Transcription 5 (STAT5) is activated through phosphorylation at tyrosine 694 in response to many cytokines and growth factors including IL-2, IL-3, GM-CSF and prolactin. Aberrant STAT5 activity is closely related to a wide range of human cancers as STAT5 is often found to be constitutively phosphorylated in cancer cells. The phosphorylated STAT5 forms homodimers or heterodimers with other STATs, and the dimers translocate to nucleus in which DNA binding/promoter induction occurs. The STAT5 induction by IL-3 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 STAT5 signaling pathway activity.
- Screen for activators or inhibitors of the STAT5 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

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should not be passaged over 16 times.

**Functional validation:**

A. Response of STAT5 Leeporter™ – Ba/F3 cells to mIL-3.

1. Harvest STAT5 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 mIL-3 and incubate cells at 37°C in a $CO_2$ incubator for 16 hours.

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

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

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

**LIMITED USE RESTRICTIONS:**

**THIS PRODUCT IS SOLELY FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**

**By use of this product, user agrees to be bound by the terms of this limited use statement.**

**This product is solely for Internal Research Purposes and not for Commercial Purposes. Commercial Purposes include, but are not limited to (1) use of the cell line in manufacturing; (2) use of the cell line to provide a service, information or data; (3) use of the cell line for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the cell line whether or not such cell lines are resold for use in research. The buyer cannot sell, give or otherwise transfer this product to a third party.**

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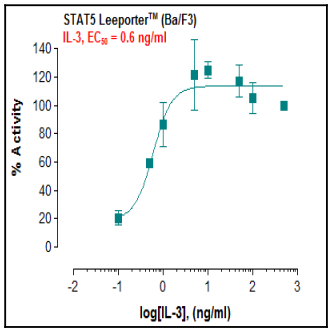



Fig-1: Induction of STAT5 activity by mIL-3 in STAT5 Leeporter™ - Ba/F3 cells.