

## 14-113ACL: STAT1 Lleeporter™ Luciferase Reporter-RAW264.7 Cell Line

**Application :** Functional Assay

### Description

The STAT1 Lleeporter™ Luciferase Reporter cell line is a stably transfected RAW264.7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the interferon (IFN) gamma activation sequence-based STAT1 response element, so that the cell line is designed to measure the transcriptional activity of STAT1. As a transcription factor, Signal Transducer and Activator of Transcription 1 (STAT1) is activated through phosphorylation at tyrosine 701 in response to various cytokines and growth factors such as IFN-alpha, IFN-gamma, IL-6, EGF and PDGF. The phosphorylated STAT1 forms homodimers or heterodimers with STAT3, and the dimers translocate to nucleus in which DNA binding/promoter induction occurs. The STAT1 induction by IFN-gamma is shown in Figure 1.

### Product Info

<b>Amount :</b>	1 Vial
<b>Content :</b>	Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.
<b>Storage condition :</b>	Immediately upon receipt, store in liquid nitrogen.

### Application Note

#### Application:

- Monitor the STAT1 signaling pathway activity.
- Screen for activators or inhibitors of the STAT1 signaling pathway.

#### Culture conditions:

Cells should be grown at 37°C with 5% CO<sub>2</sub> using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin (Note: 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<sub>2</sub> incubator.

Leave the T25 flask in the incubator for 1~2 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. **Note: RAW264.7 cells may not be detached well by trypsinization only. So you may need to use a cell scraper to harvest the trypsinized cells.**

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. To achieve satisfactory results, cells should not be passaged over 16 times.

#### Functional validation:

#### A. Response of STAT1 LEEporter™ - RAW264.7 cells to mIFN-gamma.

1. Plate STAT1 LEEporter™ - RAW264.7 cells into a white solid-bottom 96-well microplate in 100  $\mu$ l of growth medium at  $1 \times 10^5$  cells/well and incubate cells at 37°C in a CO<sub>2</sub> incubator for 4-6 hours.
2. Stimulate cells with different concentrations of mouse interferon gamma and incubate cells at 37°C in a CO<sub>2</sub> incubator for 16 hours. ,
3. Equilibrate the plate to room temperature for 10 minutes.
4. Add 50  $\mu$ l 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.

#### LIMITED USE RESTRICTIONS:

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**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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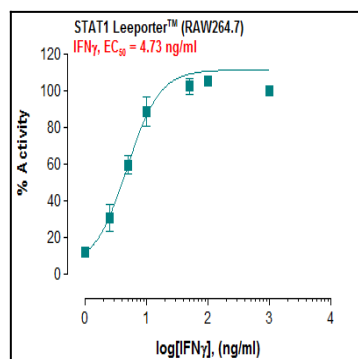


Fig-1: Induction of STAT1 activity by IFN-gamma in STAT1 LEEporter™ - RAW264.7 cells.