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Data Sheet

GAL4 Reporter (Luc)-HEK293 Recombinant Cell Line **Catalog: 60656**

Description

The *GAL4 Reporter (Luc) – HEK293 Cell Line* contains a firefly luciferase gene under the control of a multimerized GAL4 upstream activation sequence (UAS) stably integrated into HEK293 cells. The cell line does not contain any exogenous activators of the GAL4 reporter and can be used alongside BPS Cat. #60655 as a control. It can also be used for other experiments requiring stable transfection of the GAL4 reporter, including transient assays of a gene of interest fused to GAL4-DBD (DNA Binding Domain).

Format

Each vial contains $\sim 1.5 \times 10^6$ cells in 1 ml of 10% DMSO.

Mycoplasma testing

The cell line has been screened using the PCR-based Venor™ GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

Storage

Immediately upon receipt, store in liquid nitrogen.

Culture conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na-pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) and 400 µg/ml of Geneticin (Life Technologies #11811031).

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, and transfer to a tube containing 10 ml of Thaw Medium 1. Next, spin down the cells, resuspend cells in pre-warmed Thaw Medium 1, and transfer resuspended cells to a T25 flask and culture in a CO₂ incubator at 37°C overnight. The next day, replace the medium with fresh Thaw

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Medium 1, and continue growing culture in a CO₂ incubator at 37°C until the cells are ready to be split. At first passage, switch to Growth Medium 1B (**containing Geneticin**). Cells should be split before they reach complete confluence.

To passage the cells, rinse with phosphate-buffered saline (PBS) and detach cells from the culture vessel with Trypsin/EDTA. After detaching, add Growth Medium 1B and transfer to a 15 ml conical tube to spin down the cells. After spinning, resuspend cells in complete Growth Medium 1B and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly or twice a week.

Sample Protocol for Transient Transfection of GAL4 Reporter (Luc) – HEK293 cells

1. One day before transfection, seed cells at a density of ~30,000 cells per well in 100 µL of Thaw Medium 1 so that cells will be 90% confluent at the time of transfection.

2. The next day, transfect cells according to manufacturer's protocols. For a typical mammalian expression vector containing the GAL4-DBD linked to a gene of interest, use 100ng of DNA per well.

Set up each treatment in at least triplicate.

4. Incubate cells at 37°C in a 5% CO₂ incubator. After ~24 hours of transfection, change medium to 100 µL fresh Thaw Medium 1.

5. Perform luciferase assay using ONE-Step™ Luciferase Assay System (BPS cat# 60690) and following the protocol provided: Add 100 µL of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~10 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. Fold induction of GAL4 luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells

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