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Data Sheet

Glucocorticoid Receptor Pathway GR-GAL4 Reporter (Luc)-HEK293 cell Line
Catalog #: 60655

Background

The glucocorticoid signaling pathway plays an important role in development, fluid homeostasis, cognition, immune response, and metabolism. Glucocorticoids are a class of steroid hormones that bind to the glucocorticoid receptor, causing it to translocate to the nucleus. Upon translocation, the receptor can regulate the transcription of a large number of genes, including those that regulate glucose metabolism and inflammatory responses.

Description

The *Glucocorticoid Receptor Pathway GAL4 Reporter (Luc) – HEK293 Cell Line* contains a firefly luciferase gene under the control of glucocorticoid receptor ligand binding domain that is fused to the DNA binding domain (DBD) of GAL4 (GAL4 DBD-GR) stably integrated into HEK293 cells. This fusion construct activates firefly luciferase expression under the control of a multimerized GAL4 upstream activation sequence (UAS). This allows for specific detection of glucocorticoid-induced activation of the glucocorticoid receptor without the need for individual transcriptional targets and with low cross-reactivity for other nuclear receptor pathways. This cell line is validated for response to stimulation of dexamethasone and to the treatment with mifepristone, an inhibitor of the glucocorticoid signaling pathway.

Applications

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

Format

Each vial contains ~1.5 X 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.

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General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #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 1G (BPS Cat. #79544): Thaw Medium 1 (BPS Cat #60187) and 400 µg/ml of Geneticin (Life Technologies #11811031) and 50 µg/ml of Hygromycin B (Hyclone #SV3007001).

Cells should be grown at 37°C with 5% CO_2 using growth medium 1G (Thaw Medium 1 plus Geneticin and Hygromycin B).

If culturing cells in medium from other vendors, it may be required to lower the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

To thaw the cells, 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 **(no Geneticin and Hygromycin B)**. Next, spin down the cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin and Hygromycin B**), and transfer to a T25 flask. Culture the cells at 37°C in a 5% CO_2 incubator until the cells reach confluence. At first passage, switch to growth medium 1G **(contains Geneticin and Hygromycin B).** 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 1G and transfer to a 15 ml conical tube to spin down the cells. After spinning, resuspend cells in growth medium 1G and seed appropriate aliquots of cell suspension into new culture vessels.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add growth medium 1G and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with 1:8 -1:20 ratio weekly.

Functional Validation and Assay Performance

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

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Materials Required but Not Supplied for Cell Culture

- Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1G (BPS Cat. #79544)

Materials Required but Not Supplied for Cellular Assay

- Dexamethasone (Sigma #D4902)
- Mifepristone/RU-486 (Sigma #M8046)
- HEK293 assay medium: DMEM (without phenol red) (Hyclone #SH30604.01) + 10% charcoal/dextran-treated FBS (Hyclone #SH30068.02), 4 mM L-Glutamine + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- ONE-Step™ Luciferase Assay System (BPS Cat. #60690)
- Luminometer

A. Dose response of *GAL4 Reporter (Luc) – HEK293* cells to dexamethasone

1. Harvest GAL4 reporter (Luc)-HEK293 cells from culture in growth medium G1 and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Incubate cells at 37°C in a CO_2 incubator overnight (~16 hours).

3. Add 5 µl of three-fold serial dilutions of dexamethasone in assay medium to stimulated wells. Typically, 8 – 12 concentrations are used.
   Add 5 µl of assay medium to the unstimulated control wells.
   Add 50 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

4. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.

5. Perform luciferase assay using ONE-Step™ Luciferase Assay System 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 dexamethasone-stimulated well / average background-subtracted luminescence of unstimulated control wells

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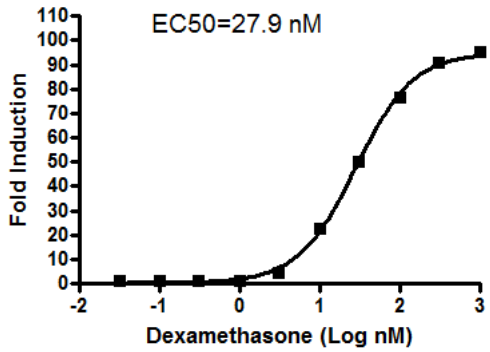
Figure 1. Dose response of GAL4 reporter (luc)-HEK293 cells to dexamethasone. The results are shown as fold induction of GAL4 luciferase reporter expression.



B. Inhibition of dexamethsone-induced reporter activity by an antagonist of the glucocorticoid signaling pathway in *GAL4 Reporter (Luc)-HEK293 cells*

1. Harvest GAL4 reporter (Luc)-HEK293 cells from culture in growth medium 1G and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Incubate cells at 37°C in a CO_2 incubator overnight (~16 hours).

3. Add 5 µl of diluted mifepristone (a glucocorticoid antagonist) in three-fold serial dilutions in assay medium to inhibited wells. Typically, 8 – 12 concentrations are used. Set up each treatment in at least triplicate. Incubate cells at 37°C in a CO_2 incubator for 1 hour.

4. Add 5 µl of 200 nM dexamethasone in assay medium to stimulated wells (final dexamethasone concentration = 20 nM).
   Add 5 µl of assay medium to the unstimulated control wells (for determining the basal activity).
   Add 55 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay System 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.*

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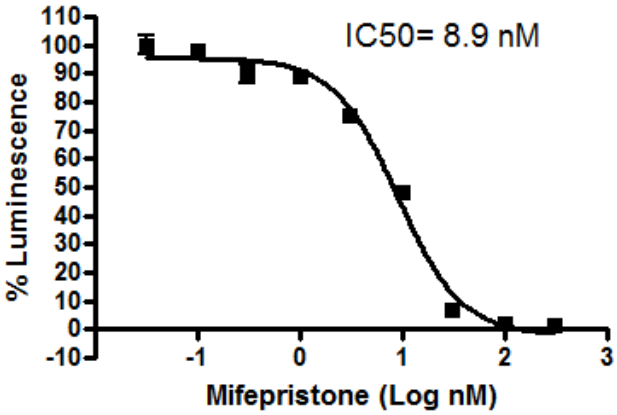
6. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 2. Inhibition dose response curve of dexamethasone-induced reporter activity by mifepristone in GAL4 reporter (Luc)-HEK293 cells. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with dexamethasone in the absence of mifepristone was set at 100%.



Reference

Paguio A, *et al*. (2010) Improved Dual-Luciferase Reporter Assays for Nuclear Receptors. *Curr Chem Genomics*. **4:** 43-49.

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