GR-GAL4 Luciferase Reporter HEK293 Cell Line (Glucocorticoid Receptor Pathway)

#60655

Description

The GR-GAL4 Luciferase Reporter HEK293 Cell Line is a HEK293 cell line expressing firefly luciferase under the control of glucocorticoid receptor ligand binding domain fused to the DNA binding domain (DBD) of GAL4 (GAL4 DBD-GR). This fusion construct activates firefly luciferase expression via control of a multimerized GAL4 upstream activation sequence (UAS). This system allows specific detection of glucocorticoid-induced activation of the glucocorticoid receptor without the need for individual transcriptional targets and has low cross-reactivity with other nuclear receptor pathways. This cell line has been validated by stimulation with dexamethasone and treatment with mifepristone, an inhibitor of the glucocorticoid signaling pathway.

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 by binding to the GRE (glucocorticoid response elements), including those that regulate glucose metabolism and inflammatory responses. The complex receptor:ligand results in up-regulation of expression of anti-inflammatory proteins (transactivation), and downregulates the expression of pro-inflammatory ones by inhibiting the translocation of the transcription factors required for their expression to the nucleus (transrepression). Glucocorticoids are used in the treatment of diseases linked to over stimulation of the immune system, for example in allergies, asthma, autoimmune diseases, and sepsis. They have also been used in cancer therapy at high doses, as they inhibit lymphocyte proliferation in lymphoma and leukemia, and organ transplant. Long term use of glucocorticoids can result in cataract, hypertension, type 2 diabetes, among other side effects. An in depth understanding of the glucocorticoid signaling pathway will lead to better tailored treatment options.

Application(s)

- Monitor glucocorticoid signaling pathway activity.
- Screen for compound activity on the glucocorticoid signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Dexamethasone	Sigma #D4902
Mifepristone/RU-486	Sigma #M8046
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended* to. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate and 1% Penicillin/Streptomycin

Growth Medium 1G (BPS Bioscience #79544):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of G418 and 50 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:

DMEM (without phenol red) + 10% charcoal/dextran-treated FBS, 4 mM L-Glutamine and 1% Pen/Strep

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 200 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent (at 90% confluency). At first passage and subsequent passages, use Growth Medium 1G.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:8-1:20 twice a week.

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

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

- The following assay was 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.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated Cells"/ "Test Compound", "Background Control" and "Unstimulated Control" conditions.

A. Dose Response of GR-GAL4 Luciferase Reporter HEK293 Cell Line to dexamethasone

1. Harvest GR-GAL4 Luciferase Reporter HEK293 cells from culture in Growth Medium 1G and seed cells at a density of 30,000 cells per well in 45 µl of Assay Medium into a clear-bottom 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate at 37°C with 5% CO_2 overnight (~16 hours).

3. Prepare a serial dilution of dexamethasone in Assay Medium at 10x the final testing concentrations (5 µl/well).

4. Add 5 µl of diluted dexamethasone to the "Stimulated Cells" wells.

5. Add 5 µl of Assay Medium to the "Unstimulated Control" wells.

6. Add 50 µl of Assay Medium to "Background Control" wells (cell-free wells).

7. Incubate at 37°C with 5% CO_2 for 24 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent per well.

9. Incubate at Room Temperature (RT) for ~10 minutes.

10. Measure luminescence using a luminometer.

11. The "Background Control" luminescence value should be subtracted from all readings.

12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

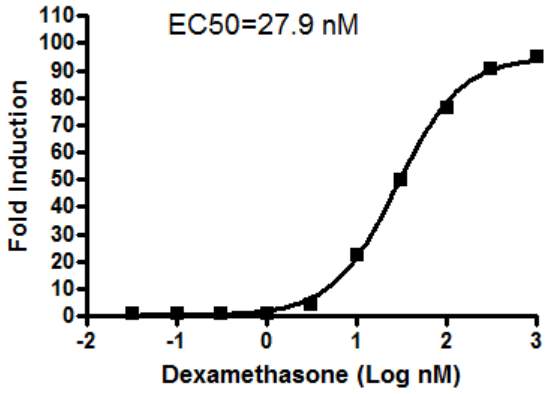

Figure 1. Dose response curve of GR-GAL4 Luciferase Reporter HEK293 Cell Line to dexamethasone. GR-GAL4 Luciferase Reporter HEK293 cells were treated with increasing concentrations of dexamethasone. The results are shown as fold induction of luciferase reporter expression. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

B. *GR-GAL4 Luciferase Reporter HEK293 Cell Line* inhibition of the dexamethsone-induced reporter activity by an antagonist of the glucocorticoid signaling pathway.

1. Harvest GR-GAL4 Luciferase Reporter HEK293 cells from culture in Growth Medium 1G and seed cells at a density of 30,000 cells per well in 45 µl of Assay Medium into clear-bottom 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").
2. Incubate at 37°C with 5% CO_2 overnight (~16 hours).
3. Prepare a three-fold serial dilution of mifepristone in Assay Medium at 10x the final testing concentrations (5 µl/well).
4. Add 5 µl of diluted mifepristone to the "Test Compound" wells.
5. Incubate at 37°C with 5% CO_2 for 1 hour.
6. Prepare a 200 nM dexamethasone solution in Assay Medium (5 µl/well).
7. Add 5 µl of dexamethasone solution to the "Test Compound" wells.
8. Add 10 µl of Assay Medium to the "Unstimulated Control" wells.
9. Add 55 µl of Assay Medium to "Background Control" wells (cell-free wells).
10. Incubate at 37°C with 5% CO_2 for 24 hours.
11. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.
12. Incubate at RT for ~10 minutes.

13. Measure luminescence using a luminometer.

14. The "Background Control" luminescence value should be subtracted from all readings.

15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of the test compound wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Test\ Compound\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

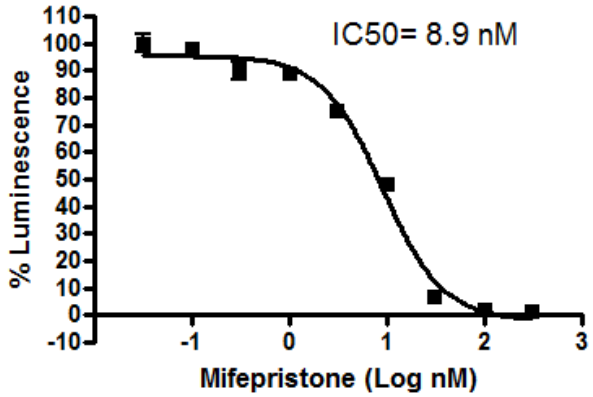

Figure 2. Dose response curve of GR-GAL4 Luciferase Reporter HEK293 Cell Line dexamethasone-induced-reporter activty to mifepristone.
GR-GAL4 Luciferase Reporter HEK293 cells were treated with increasing concentrations of miferpristone. The results are shown as fold induction of luciferase reporter expression. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References:
Paguio A, *et al*., 2010 *Curr Chem Genomics*. 4: 43-49.

Related Products

Products	*Catalog #*	*Size*
Transfection Collection™: GAL4 Transient Pack Glucocorticoid Receptor Pathway	79265	100 reactions
GAL4 Luciferase Reporter HEK293 Cell Line	60656	2 vials
GAL4 Reporter Kit (Glucocorticoid Receptor Pathway)	60522	2 vials
GAL4 DBR-GR Lentivirus	78632	500 µl x 2
GR-GAL Luciferase Reporter Jurkat Cell Line	78632	2 vials