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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The *Glucocorticoid Receptor (GR)-GAL4 Luciferase Reporter Jurkat Cell Line* contains an engineered transcription factor stably integrated into the genome of Jurkat cells, which consists of the glucocorticoid receptor ligand binding domain fused to the DNA binding domain of GAL4. This fusion construct activates firefly luciferase expression which is 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.

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 regulates the transcription of a large number of genes, including those that regulate glucose metabolism and inflammatory responses.

Application

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

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, suspension

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium 2C	BPS Bioscience #78544
Dexamethasone	Cayman #11015
Mifefristone	Cayman #10006317
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in 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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37˚C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI-1640 medium supplemented with 10% FBS and 1% Penicillin and Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin and Streptomycin plus 1000 µg/ml of G418 and 200 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium 2C (BPS Bioscience #78544):
RPMI-1640 medium (no phenol red) supplemented with 10% charcoal-stripped FBS and 1% Penicillin and Streptomycin.

Cell Culture Protocol

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 2 (no Geneticin or Hygromycin).
   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 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (no Geneticin or Hygromycin).

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 viability. For a T25 flask, add 3-4 ml of Thaw Medium 2 (no Geneticin or Hygromycin), and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged when they reach a density of 2 - 3 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A (contains Geneticin and Hygromycin).

Cell Passage

This cell line grows slower than the parental Jurkat cells. Dilute the cell suspension into new culture vessels when they reach a density of 2 -3 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2A (contains Geneticin and Hygromycin). The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2-3 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.

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

3. Transfer the vials to liquid nitrogen the next day for storage.

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

Functional characterization of GR-GAL4 Luciferase Reporter Jurkat Cell Line

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

Assay Medium: Assay Medium 2C (BPS Bioscience #78544)

A. Dose response of GR-GAL4 Luciferase Reporter Jurkat cell line to dexamethasone

1. Culture the cells so that they reach a density of 1.5~ 2.5 x 10^6 cells/ml on the day of the experiment.
Note: Cell density should be >1.5 x 10^6/ml. You may not want to count the cells at this step unless the cells are fully confluent. If you split the fully confluent cells at 1:6 or 1:7 ratio and grow the cells for 1 week without agitation, you will see the medium color is changed to yellow and big cell clumps under the microscope. If you are not sure if the cell density is higher than 1.5 x 10^6*/ml, wait one more day and proceed to the next steps*.

2. On the day of the experiment, prepare serial dilutions of Dexamethasone in PBS at concentrations 10-fold higher than the desired final concentrations, right before harvesting the cells (*for example, if the highest desired concentration of Dexamethasone is 100 nM, prepare 100 µM Dexamethasone in DMSO. Then dilute 100 µM Dexamethasone 100-fold in PBS, resulting in 1 µM Dexamethasone. The concentration of DMSO is now 1% in PBS).*

3. Harvest the cells by centrifugation at 300 x g for 5 min.

4. Resuspend the cells in Assay Medium 2C using the same volume as the original culture volume (if you harvested 10 ml of the cell culture, resuspend the cells in 10 ml of Assay Medium 2C) and count the cells.

5. Cell density should be 1.5 - 2.5 x 10^6 cells/ml.

6. Dilute the cells with Assay Medium 2C to prepare the cells at a density of ~ 800,000 cells/ml.

7. Add 90 µl of the prepared cells to each well and add 10 µl of the 10-fold intermediate dilutions of Dexamethasone.

8. For the negative control/background, add 10 µl of PBS containing 1% DMSO.

9. Incubate the cells for 5 to 5.5 hours in a 5% CO_2 incubator at 37°C.

10. After the incubation, perform the luciferase assay using the ONE™-Step Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of the ONE-Step Luciferase reagent per well, rock at room temperature for ~ 15 minutes, and read the luminescence.

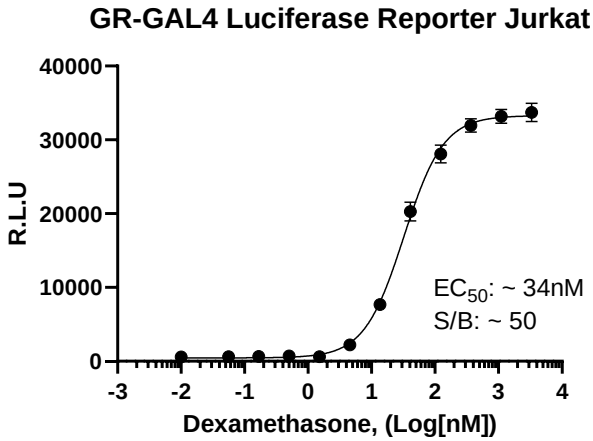

Figure 1. Dose response of Dexamethasone in the GR-GAL4 Luciferase Reporter Jurkat Cell Line
Cells were incubated with increasing concentrations of Dexamethasone for 5 hours. The luciferase assay was performed using the ONE-Step Luciferase Assay System (BPS Bioscience #60690).

B. Inhibition of Dexamethasone-induced reporter activity by Mifepristone in GR-GAL4 Luciferase Reporter Jurkat cell line

1. Culture the cells so that they reach a density of 1.5~ 2.5 x 10^6 cells/ml on the day of the experiment.

   Note: Cell density should be >1.5 x 10^6/ml. You may not want to count the cells at this step if the cells are not fully confluent. If you split the fully confluent cells at 1:6 or 1:7 ratio and grow the cells for 1 week without agitation, you will see the medium color is changed to yellow and big cell clumps under the microscope. If you are not sure if the cell density is higher than 1.5 x 10^6/ml, wait one more day and proceed to the next steps.

2. On the day of the experiment, prepare serial dilutions of Mifepristone in Assay Medium 2C at concentrations 10-fold higher than the desired final concentrations, right before harvesting the cells (for example if the highest desired concentration of Mifepristone is 100 nM, prepare 100 µM Mifepristone in DMSO. Then dilute 100 µM Mifepristone 100-fold in Assay Medium 2C, resulting in 1 µM Mifepristone. The concentration of DMSO is now 1% in Assay Medium 2C).

3. Harvest the cells by centrifugation at 300 x g for 5 min.

4. Resuspend the cells in Assay Medium 2C using the same volume as the original culture volume (if you harvested 10 ml of the cell culture, resuspend the cells in 10 ml of the Assay Medium 2C) and count the cells.

5. Cell density should be 1.5 - 2.5 x 10^6 cells/ml.

6. Dilute the cells with Assay Medium 2C to a density of ~ 800,000 cells/ml.

7. Add 80 µl of the prepared cells to each well and add 10 µl of the intermediate dilutions of Mifepristone prepared at step 2. (For the positive control, add 10 µl of 1% DMSO in Assay Medium 2C).

8. Incubate the cells for 1 hours in a 5% CO_2 incubator at 37°C.

9. Prepare 200 µM Dexamethasone in DMSO. Then dilute 200 µM Dexamethasone 100-fold in PBS to obtain 2 µM Dexamethasone (the concentration of DMSO is now 1% in PBS).

10. After the 1-hour incubation with Mifepristone, add 10 µl of 2 µM Dexamethasone to each well.

11. For the negative control, add 10 µl of 1% DMSO in PBS.

12. Incubate the cells for 5 to 5.5 hours in a 5% CO_2 incubator at 37°C.

13. After the incubation with Dexamethasone, perform the luciferase assay using the ONE™-Step Luciferase Assay System. Add 100 µL of the ONE-Step Luciferase reagent per well, rock at room temperature for ~15 minutes, and read the luminescence.

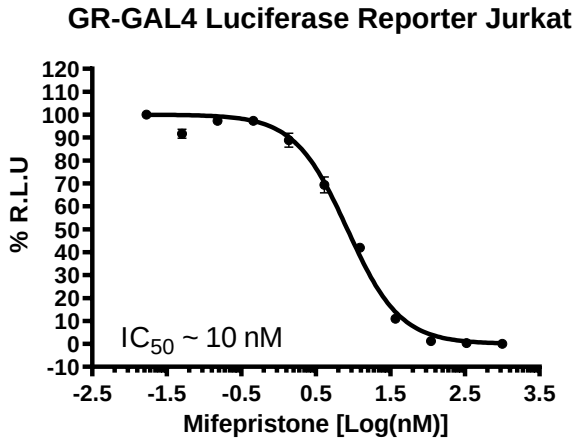

Figure 2. Dose response of Mifepristone in GR-GAL4 Luciferase Reporter Jurkat Cell Line.
GR-GAL4 Luciferase Reporter cells were incubated with various concentrations of Mifepristone for 1 hour followed by glucocorticoid signaling stimulation using 200 nM Dexamethasone for 5 hours. Luciferase assay was performed by using the One-Step Luciferase Assay System (BPS Bioscience #60690).

References

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

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Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Transfection Collection™: GAL4 Transient Pack Glucocorticoid Receptor Pathway	79265	100 reactions
GAL4 Reporter Kit (Glucocorticoid Receptor Pathway)	60522	500 reactions
GR-GAL4 Reporter (Luc)-HEK293 Recombinant Cell Line (GR Pathway)	60655	2 vials
GITR:GITRL TR-FRET Assay Kit	79054	384 reactions