SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line expresses firefly luciferase under the control of the GAL4 upstream activation sequence (UAS). It has constitutive expression of human peroxisome proliferator-activated receptor γ ligand binding domain (PPARγ LBD, amino acids 173-475) fused to the DNA binding domain (DBD) of GAL4 (GAL4 DBD). This system allows specific measurement of the effect of PPARγ agonists with low cross-reactivity from other nuclear receptors or from other PPAR isoforms.

This cell line has been validated by stimulation with Rosiglitazone, and for its lack of stimulation to GW7647 and GW501516.

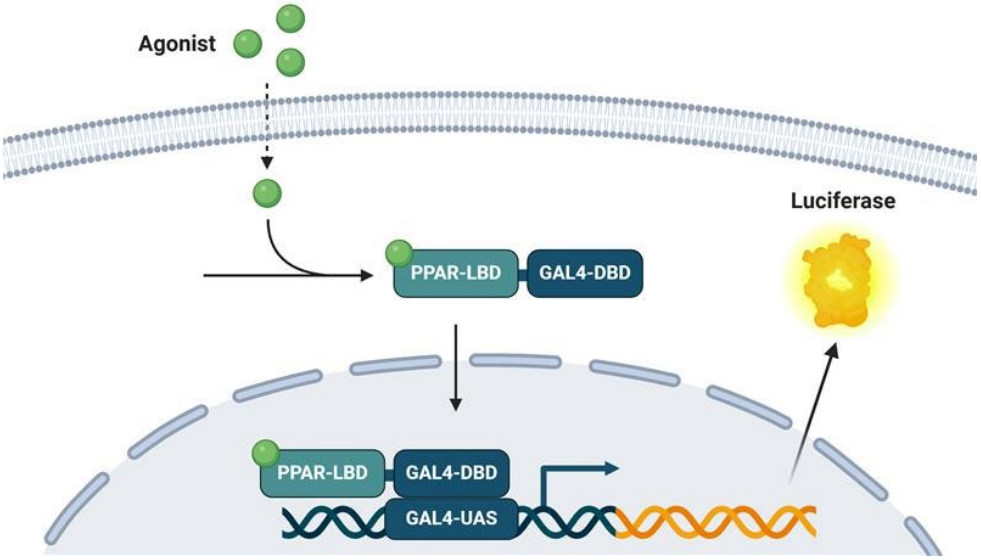

Figure 1: Illustration of the mechanism leading to luciferase induction upon agonist treatment in PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line.

Background

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors (NRs) that are important in cell differentiation and various metabolic processes. PPARγ, also known as glitazone reverse insulin resistance receptor or NR1C3 (nuclear receptor subfamily 1, group C, member 3) or PPARG, has two isoforms. PPARγ1 is found in several tissues, while isoform 2 is normally restricted to the adipose tissue. It is involved in fatty acid storage and in the expression of hormones linked to glucose homeostasis. During the last few decades, the therapeutic potential of their synthetic agonists on various metabolic diseases, including dyslipidemia, hyperglycemia, Type 2 Diabetes (T2D), as well as liver diseases, has been well-documented. Most recently, the US FDA approved Livdelzi® (seladelpar) for primary biliary cholangitis (PBC). This approval highlights the importance of the pharmacological actions of PPAR agonists.

Application(s)

Screen for specific PPARγ agonists.

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)

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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 1M	BPS Bioscience #79723

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium 6B	BPS Bioscience #82202
Rosiglitazone	Cayman #71740
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
384-well tissue culture treated white clear-bottom assay plate	PerkinElmer #6007680
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*. Note that using similar but not BPS Bioscience validated reagents can result in suboptimal performance.

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°C 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 1M (BPS Bioscience #79723):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of G418, and 0.5 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Assay Medium 6B:
DMEM (without phenol red) medium supplemented with 2% charcoal treated FBS, 1% Gluta-Max™, and 1% Penicillin/Streptomycin.

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

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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 1.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. 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.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1M.

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 1M 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 1M.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 every 3-4 days.

Cell Freezing

1. Aspirate the medium, wash the cells with 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 1M 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 1~2 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/384-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", "Background Control" and "Unstimulated Control" conditions.

A. Dose Response of PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line to Rosiglitazone (96-well)

1. Seed cells at a density of 30,000 ~ 40,000 cells per well in 90 µl of Assay Medium 6B into a white clear-bottom 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare a serial dilution of Rosiglitazone in Assay Medium 6B at 10x the final testing concentrations (10 µl/well). *e.g. Prepare a 1 mM Rosiglitazone stock solution in DMSO and dilute it to 10 µM by mixing 10 µl of 1 mM Rosiglitazone in DMSO with 990 µl of Assay Medium 6B. This 10 µM solution (containing 1% DMSO) would result in a 1 µM Rosiglitazone testing concentration. Use Assay Medium 6B containing 1% DMSO as diluent solution if preparing a serial dilution starting from the 10 µM solution.*

   Note: This cell line can tolerate 0.1% DMSO.

3. Add 10 µl of diluted Rosiglitazone to the "Stimulated Cells" wells.

4. Add 10 µl of Assay Medium 6B containing 1% DMSO to the "Unstimulated Control" wells.

5. Add 100 µl of Assay Medium 6B to "Background Control" wells (cell-free wells).

6. Incubate at 37°C with 5% CO_2 for 16 ~ 24 hours.

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

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

9. Measure luminescence using a luminometer.

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

11. 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.

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

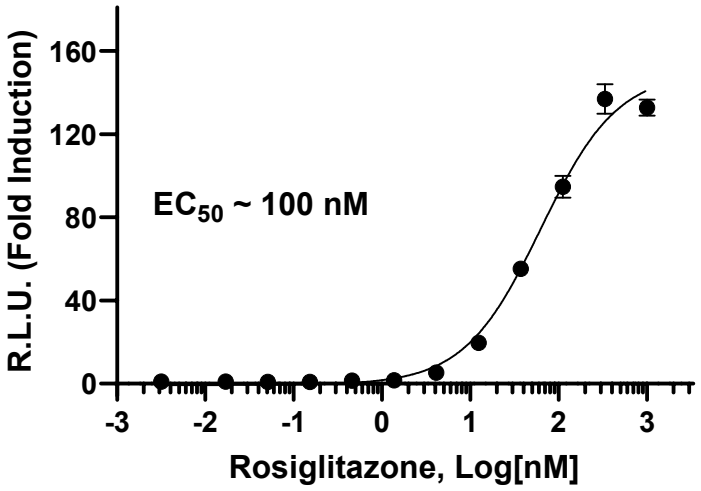

Figure 2. Dose response curve of PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line to Rosiglitazone (96-well format).
PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 cells were treated with increasing concentrations of Rosiglitazone in a 96-well plate format. Luciferase activity was measured using 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. Dose Response of PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line to Rosiglitazone (384-well)

1. Seed cells at a density of ~5,000 cells per well in 45 µl of Assay Medium 6B into a white clear-bottom 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare a serial dilution of Rosiglitazone in Assay Medium 6B at 10x the final testing concentrations as described above (step **A**-2) (5 µl/well).

3. Add 5 µl of diluted Rosiglitazone to the "Stimulated Cells" wells.
4. Add 5 µl of Assay Medium 6B containing 1% DMSO to the "Unstimulated Control" wells.
5. Add 50 µl of Assay Medium 6B to "Background Control" wells (cell-free wells).
6. Incubate at 37°C with 5% CO_2 for 16 ~ 24 hours.
7. Add 50 µl of ONE-Step™ Luciferase reagent per well.
8. Incubate at Room Temperature (RT) for ~10 minutes.
9. Measure luminescence using a luminometer.
10. The "Background Control" luminescence value should be subtracted from all readings.
11. 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.

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

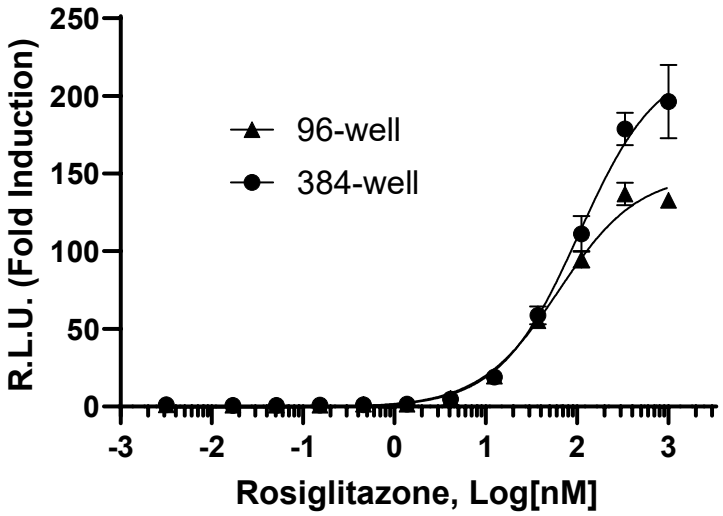

Figure 3. Dose response curve of PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line to Rosiglitazone (384-well format).
PPARγ-GAL4 Luciferase Reporter HEK293 cells were treated with increasing concentrations of Rosiglitazone in a 384-well plate. Luciferase 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) and represent a comparison of two different plate formats.

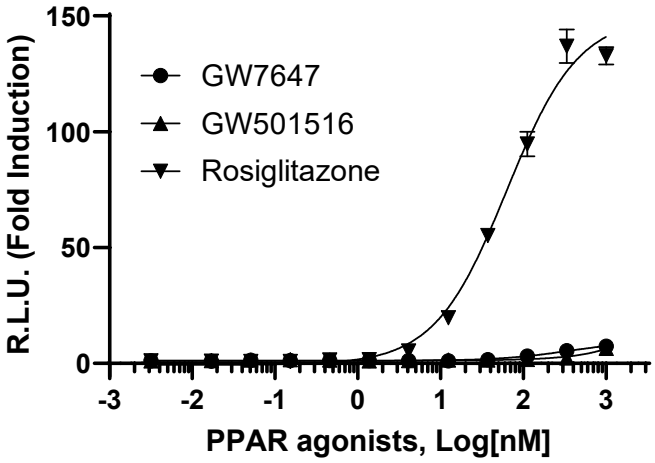

Figure 4. Selective activation of PPARγ (Peroxisome proliferator-activated receptor gamma)-GAL4 Luciferase Reporter HEK293 Cell Line.
PPARγ-GAL4 Luciferase Reporter HEK293 cells were treated with increasing concentrations of the listed PPAR agonists in a 96-well plate format. All agonists were prepared in DMSO at 1 mM and diluted in Assay Medium at 10x the final testing concentration as described in the protocol. Luciferase activity was measured using 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.

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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.

References
Berger J. P., *et al*., 2005 *Trends in Pharmacological Sciences* 5: 244-251.
Ahmadian M., *et al.*, 2013 *Nat. Med.* 19 (5): 10.1038.

Related Products

Products	*Catalog #*	*Size*
PPARα (Peroxisome proliferator-activated receptor alpha)-GAL4 Luciferase Reporter HEK293 Cell Line	82837	2 vials
TRβ-GAL4 Luciferase Reporter HEK293 Cell Line	82175	2 vials
TRα-GAL4 Luciferase Reporter HEK293 Cell Line	82633	2 vials
GAL4 Luciferase Reporter HEK293 Cell Line	60656	2 vials
GAL4 DBR-GR Lentivirus	78632	500 µl x 2
GR-GAL Luciferase Reporter Jurkat Cell Line	78632	2 vials

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