Description

GDF15 Responsive Luciferase Reporter HEK293 Cell Line is an engineered HEK293 cell line that expresses firefly luciferase under the control of Serum Response Element (SRE) in addition to the human GFRAL (GDNF family receptor alpha like) and RET complex (hGFRAL NM_207410.2 and hRET NM_020975.6). GDF15 (Growth Differentiation Factor 15) activity can be monitored by measuring luciferase activity.

The functionality of the GDF15 Responsive Luciferase Reporter HEK293 Cell Line was validated in dose-response assays using a recombinant human GDF15 and inhibition assays using an anti-GFRAL antibody as well as an anti-GDF15 antibody, Visugromab.

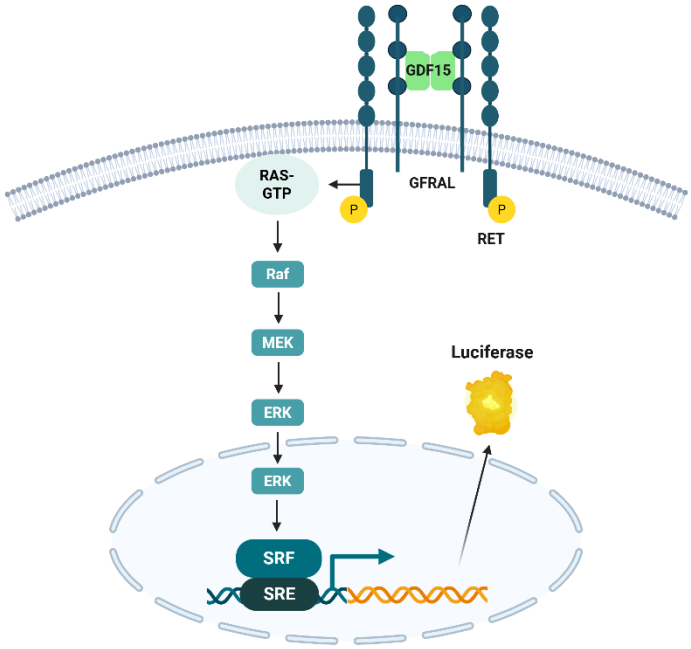

Figure 1. Illustration of the mechanism of action in the GDF15 Responsive Luciferase Reporter HEK293 Cell Line.

Background

GDF15 (Growth Differentiation Factor 15), a member of the TGF-β (tumor growth factor) superfamily, is a stress-induced cytokine that regulates cellular homeostasis, appetite, and inflammation. It also plays a crucial role in cancer-associated cachexia and tumor cell growth. Due to its involvement in various metabolic processes, significant efforts are underway to develop reagents that can antagonize or agonize the GDF15 and its signaling pathway.

Application(s)

- Screen and characterize GDF15 analogs.
- Validate anti-GDF15 antibodies and anti-GFRAL antibodies.

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)

Host Cell

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.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1M	BPS Bioscience #79723
Cell Freezing Medium	BPS Bioscience #79796

Materials Required for Cellular Assay

Name	Ordering Information
GDF15	
Anti-GFRAL antibody	BPS Bioscience #101351
Visugromab	BPS Bioscience #84017
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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 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, 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, 0.5 µg/ml of Puromycin and 400 µg/ml of Geneticin.

Media Required for Functional Cellular Assay

Assay Medium: Thaw Medium 1

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 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.

Note: Recovery of the frozen cells can take longer than a week. Change medium to fresh Thaw Medium 1 after a week.

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 the 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 4-5 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 at least 10 vials at an early passage for future use.

Functional Validation

- The following assays are designed for 96-well. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- Assay A should include "Cell-Free Control", "Unstimulated Control" and "Stimulated" conditions.
- Assay B and C should include "Cell-Free Control", "Positive Control" (GDF15, no antibody), "Negative Control" (no GDF15, no antibody) and "Test Antibody" conditions.

Assay Medium: Thaw Medium 1

A. Dose-response of GDF15 Responsive Luciferase Reporter HEK293 Cell Line to recombinant human GDF15 (96-well format)

Day 1:

1. Cells should be at ~ 70% confluency *(avoid using fully confluent cells)* before the experiment.

2. Seed GDF15 Responsive Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 35,000 to 40,000 cells per well in 90 µl of Assay Medium (Thaw Medium 1). Leave a few empty wells to determine the background luminescence ("Cell-Free Control").

3. Incubate cells at 37°C in a CO_2 incubator overnight.

Day 2:

1. Prepare a serial dilution of recombinant human GDF15 at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

2. Add 10 µl of each GDF15 dilution to the "Stimulated" wells.

3. Add 10 µl of Assay Medium to the "Unstimulated Control" (negative control) wells.

4. Add 100 µl of Assay Medium to the "Cell-Free Control" wells (for determining background luminescence).

5. Incubate the plate at 37°C in a CO_2 incubator for ~ 5 hours.

6. Add 100 µl of the ONE-Step™ Luciferase reagent to each well.

7. Rock gently at Room Temperature (RT) for ~15 minutes.

8. Measure luminescence using a luminometer.

Data Analysis

Subtract the average background luminescence (cell-free wells) from the luminescence reading of all wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - average\ background)}{(average\ luminescence\ of\ unstimulated\ cells - average\ background)}$$

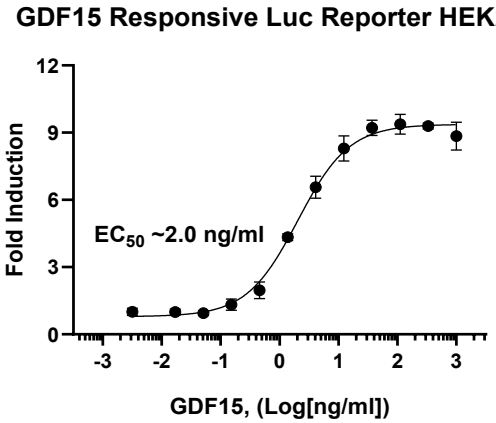

Figure 2. Dose response curve of GDF15 Responsive Luciferase Reporter HEK293 Cell Line to recombinant human GDF15 in a 96-well assay format.
Cells were treated with increasing concentrations of GDF15 in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System (#60690). Results are expressed as fold induction compared to the unstimulated control.

B. Dose-response of GDF15 Responsive Luciferase Reporter HEK293 Cell Line to an anti-GFRAL antibody

Day 1:

1. Cells should be at ~ 70% confluency (*avoid using fully confluent cells*) before the experiment.

2. Seed GDF15 Responsive Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 35,000 to 40,000 cells per well in 80 µl of Assay Medium (Thaw Medium 1). Leave a few empty wells to determine the background luminescence ("Cell-Free Control").

3. Incubate cells at 37°C in a CO_2 incubator overnight.

Day 2:

1. Prepare a serial dilution of anti-GFRAL neutralizing antibody (e.g. #101351) at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).
2. Add 10 µl of the serially diluted antibody to the "Test Antibody" wells.
3. Add 10 µl of Assay Medium to the "Positive Control" and "Negative Control" wells.
4. Incubate the plate for 1 hour at 37°C in a CO_2 incubator.
5. After 1 hour, dilute GDF15 in Assay Medium to a concentration 10-fold higher than the desired testing concentration (e.g. if the EC_{90} ~ 10 ng/ml, prepare a 100 ng/ml GDF15 solution in Assay Medium) (10 µl/ well).
6. Add 10 µl of diluted GDF15 to the "Positive Control" and "Test Antibody" wells.
7. Add 10 µl of Assay Medium to "Negative Control" wells.
8. Add 100 µl of Assay Medium to the empty wells for "Cell-Free Control" (Blank).
9. Incubate cells at 37°C in a CO_2 incubator for 5-6 hours.
10. After 5-6 hours, add 100 µl of the ONE-Step™ Luciferase reagent to each well.
11. Rock gently at RT for ~15 minutes.
12. Measure luminescence using a luminometer.

Data Analysis

Subtract the average luminescence of the "Negative Control" wells (no GDF15, no antibody) from the luminescence reading of all wells. The percent luminescence is the average negative control-subtracted luminescence of the antibody-treated wells divided by the average negative control-subtracted luminescence of the "Positive Control" wells (GDF15 only, no antibody) multiplied by 100.

$$\%\ Luminescence = \left(\frac{(luminescence\ of\ Test\ Antibody\ cells - average\ Negative\ Control)}{(average\ luminescence\ of\ Positive\ Control - average\ Negative\ Control)} \right) X100$$

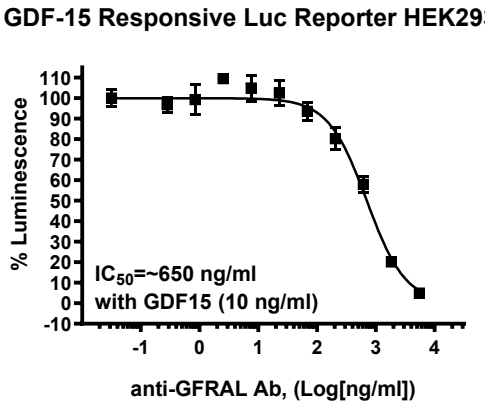

Figure 3. Inhibition of GDF15-induced reporter activity by an anti-GFRAL neutralizing antibody in GDF15 Responsive Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of an anti-GFRAL neutralizing antibody as described in the protocol and incubated for 1 hour in a CO_2 incubator prior to stimulation with GDF15 (10 ng/ml) for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as % luminescence as described in the equation above.

C. Dose-response of GDF15 Responsive Luciferase Reporter HEK293 Cell Line to an anti-GDF15 antibody

Day 1:

1. Cells should be at ~ 70% confluency (*avoid using fully confluent cells*) before the experiment.

2. Seed GDF15 Responsive Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 35,000 to 40,000 cells per well in 80 µl of Assay Medium (Thaw Medium 1). Leave a few empty wells to determine the background luminescence ("Cell-Free Control").

3. Incubate cells at 37°C in a CO_2 incubator overnight.

Day 2:

1. Prepare a serial dilution of anti-GDF15 antibody (e.g. Visugromab, #84017) at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

2. In an empty assay plate, add 10 µl of diluted anti-GDF15 antibody to the "Test Antibody" wells.

3. To this assay plate, add 10 µl of Assay Medium to the "Positive Control" (no antibody, GDF15) and "Negative Control" (no antibody, no GDF15)" wells.

4. Prepare a dilution of GDF15 at 100 ng/ml in Assay Medium (10 µl/ well).

5. Add 10 µl of this dilution to the "Positive Control" and to the "Test antibody" wells. The final concentration of GDF15 in the reaction will be 10 ng/ml.

6. Add 10 µl of Assay Medium only to "Negative Control" wells.

7. Incubate this plate for 1 hour at RT.

8. After 1 hour, transfer 20 µl of the mixtures prepared to the wells containing the appropriate cell-containing wells.

9. Add 100 µl of Assay Medium to the empty wells for "Cell-Free Control".

10. Incubate the cells at 37°C in a CO_2 incubator for 5-6 hours.

11. After 5-6 hours, add 100 µl of the ONE-Step™ Luciferase reagent to each well.

12. Rock gently at RT for ~10 minutes.

13. Measure luminescence using a luminometer.

Data Analysis

Subtract the average luminescence of the "Negative Control" wells (no GDF15, no antibody) from the luminescence reading of all wells. The % luminescence is the average negative control-subtracted luminescence of the antibody treated wells divided by the average negative control-subtracted luminescence of the "Positive Control" wells (GDF15 only, no antibody) multiplied by 100.

$$\%\ Luminescence = \left(\frac{(luminescence\ of\ Test\ Antibody\ cells - average\ Negative\ Control)}{(average\ luminescence\ of\ Positive\ Control - average\ Negative\ Control)}\right) X100$$

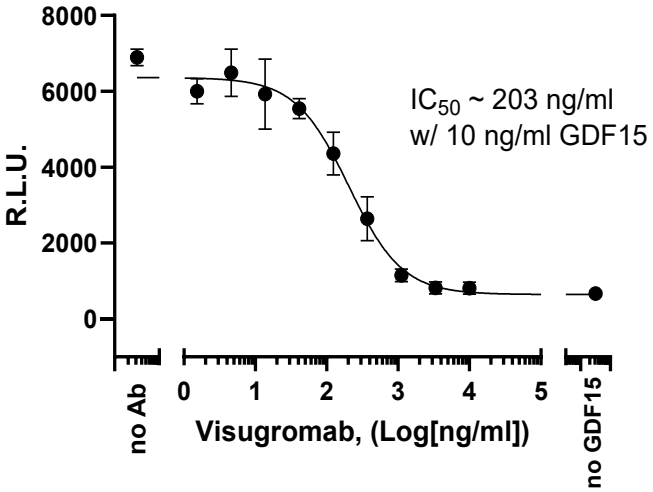

Figure 4. Inhibition of GDF15 induced reporter activity by an anti-GDF15 neutralizing antibody in GDF15 Responsive Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of anti-GDF15 neutralizing antibody as described in the protocol and incubated for 5-6 hours in a CO_2 incubator. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as % luminescence as described in the equation above.

Data shown is representative.

Sequence
Human GFRAL sequence (NM_207410.2)

MIVFIFLAMGLSLENEYTSQTNNCTYLREQCLRDANGCKHAWRVMEDACNDSDPGDPCKMRNSSYCNLSIQYLVESNFQFKECL
CTDDFYCTVNKLLGKKCINKSDNVKEDKFKWNLTTRSHHGFKGMWSCLEVAEACVGDVVCNAQLASYLKACSANGNPCDLKQC
QAAIRFFYQNIPFNIAQMLAFCDCAQSDIPCQQSKEALHSKTCAVNMVPPPTCLSVIRSCQNDELCRRHYRTFQSKCWQRVTRKC
HEDENCISTLSKQDLTCSGSDDCKAAYIDILGTVLQVQCTCRTITQSEESLCKIFQHMLHRKSCFNYPTLSNVKGMALYTRKHANKI
TLTGFHSPFNGEVIYAAMCMTVTCGILLLVMVKLRTSRISSKARDPSSIQIPGEL

Human RET sequence (NM_144701.3)

MAKATSGAAGLRLLLLLLLPLLGKVALGLYFSRDAYWEKLYVDQAAGTPLLYVHALRDAPEEVPSFRLGQHLYGTYRTRLHENNWI
CIQEDTGLLYLNRSLDHSSWEKLSVRNRGFPLLTVYLKVFLSPTSLREGECQWPGCARVYFSFFNTSFPACSSLKPRELCFPETRPSF
RIRENRPPGTFHQFRLLPVQFLCPNISVAYRLLEGEGLPFRCAPDSLEVSTRWALDREQREKYELVAVCTVHAGAREEVVMVPFPV
TVYDEDDSAPTFPAGVDTASAVVEFKRKEDTVVATLRVFDADVVPASGELVRRYTSTLLPGDTWAQQTFRVEHWPNETSVQAN
GSFVRATVHDYRLVLNRNLSISENRTMQLAVLVNDSDFQGPGAGVLLLHFNVSVLPVSLHLPSTYSLSVSRRARRFAQIGKVCVEN
CQAFSGINVQYKLHSSGANCSTLGVVTSAEDTSGILFVNDTKALRRPKCAELHYMVVATDQQTSRQAQAQLLVTVEGSYVAEEA
GCPLSCAVSKRRLECEECGGLGSPTGRCEWRQGDGKGITRNFSTCSPSTKTCPDGHCDVVETQDINICPQDCLRGSIVGGHEPGE
PRGIKAGYGTCNCFPEEEKCFCEPEDIQDPLCDELCRTVIAAAVLFSFIVSVLLSAFCIHCYHKFAHKPPISSAEMTFRRPAQAFPVSY
SSSGARRPSLDSMENQVSVDAFKILEDPKWEFPRKNLVLGKTLGEGEFGKVVKATAFHLKGRAGYTTVAVKMLKENASPSELRDL
LSEFNVLKQVNHPHVIKLYGACSQDGPLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALTMGDLISFAWQIS
QGMQYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVYEEDSYVKRSQGRIPVKWMAIESLFDHIYTTQSDVWSFGVLLW
EIVTLGGNPYPGIPPERLFNLLKTGHRMERPDNCSEEMYRLMLQCWKQEPDKRPVFADISKDLEKMMVKRRDYLDLAASTPSDS
LIYDDGLSEEETPLVDCNNAPLPRALPSTWIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPNDSVYANWMLSPSAAKLM
DTFDS

References
Hsu J.-Y., *et al.*, 2017 *Nature* 550: 255-259.
Tsai V., *et. al.*, 2018 *Cell Metabolism* 28(3):353-368.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
ChemiVerse™ RET Kinase Assay Kit	82575	96 reactions
Ret, GST-tag Recombinant	40267	10 µg
GDF15: GFRAL [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit	82875	96 reactions

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