Description

The Activin E Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express human Activin A Receptor Type 1C (ACVR1C), also known as Activin Receptor-Like Kinase 7 (ALK7) (NM_145259.3). The construct was delivered by lentiviral transduction of TGFβ/Activin A/Myostatin-Responsive Reporter HEK293 Cell Line (#60653), which expresses a firefly luciferase reporter driven by SMAD-responsive elements (SMAD binding elements, SBE). After activation by Activin E, the endogenous SMAD transcription factors bind to the response elements, inducing transcription of the luciferase reporter.

This cell line has been validated to respond to Activin E.

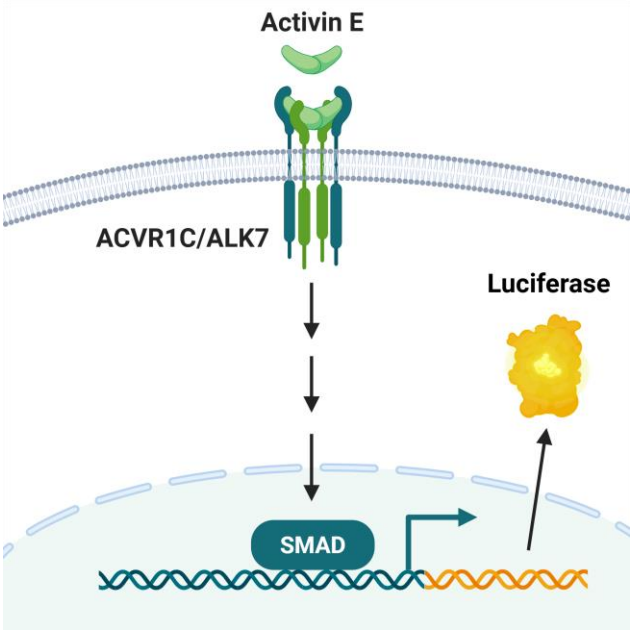

Figure 1: Illustration of the mechanism of action of the Activin E Responsive Luciferase Reporter HEK293 Cell Line.

Background

Activin Receptor-Like Kinase 7 (ALK7), also known as Activin Receptor Type 1C (ACVR1C), is a type I serine/threonine kinase receptor that belongs to the TGF-β (transforming growth factor beta) superfamily, which plays an important role in regulating fat storage and metabolism. It is found in reproductive tissue, pancreatic cells, brain cells, and adipose tissue where it is highly expressed. ALK7 signaling is carried out via a tetrameric complex consisting of two TGF-β type II receptors (ActIIA or ActIIB) and two type I receptors (ALK7). Binding of Activin E results in phosphorylation of type II receptors followed by activation of ALK7 kinase activity. Once activated, ALK7 phosphorylates the intracellular signaling proteins SMAD2/3 (SMAD family member 2/3). Nuclear translocation leads to interaction with transcription factors to control the expression of target genes. In obesity, elevated ALK7 signaling has been linked to decreased lipolysis and lipid oxidation, leading to increased fat storage. Furthermore, genetic studies have indicated that decreased ALK7 activity is associated with reduced abdominal adipose tissue and enhanced insulin sensitivity. The development of therapeutic strategies designed to modulate ALK7 is a growing field of research showing promising results for the treatment of metabolic diseases.

Application

- Screen and characterize modulators of ALK7 signaling.

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 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 1	BPS Bioscience #60187
Growth Medium 1M	BPS Bioscience #79723

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Recombinant human activin E PLUS™ protein	Qkine #Qk067
A 83-01	BPS Bioscience #84236
White, clear-bottom cell culture plate, 96-well	Corning #3610
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(s) 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 plus 400 µg/ml of Geneticin and 0.5 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 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. 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 300 *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 or T75 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 pass.

5. 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 1:6 to 1:12 once or twice a week.

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.

4. Remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

5. 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.

6. 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 a 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay conditions should be performed in triplicate.
- Assay A should include “Cell-Free Control”, “Stimulated”, and “Unstimulated Control” conditions.
- Assay B should include “Cell-Free Control”, “Unstimulated, No Compound”, “Stimulated, No Compound”, and “Stimulated, Test Compound” conditions.

Assay Medium: Thaw Medium 1.

A. Dose-response of Activin E Responsive Luciferase Reporter HEK293 Cell Line to an Agonist

1. Seed Activin E Responsive Luciferase Reporter HEK293 cells into a white clear-bottom 96-well cell culture plate at a density of ~30,000 cells per well in 90 µl of Assay Medium. Leave a few empty wells to determine the background luminescence (“Cell-Free Control”).

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

3. The next day, prepare a serial dilution of agonist at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

   3.1. For agonists soluble in water-based buffers, prepare a serial dilution in Assay Medium 10-fold higher than the desired final concentration.

   Assay Medium is the Diluent Solution.

   OR

   3.2 For agonists soluble in HCl prepare a stock solution in 100% HCl at a concentration 1,000x higher than the highest desired final concentration, then dilute it to 10-fold higher than the desired final concentration with Assay Medium. The concentration of HCl is now 1%.

   Prepare a serial dilution using Assay Medium containing 1% HCl.

   For controls use Assay Medium with 1% HCl as Diluent Solution.

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

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

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

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

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

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

10. 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 activity is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

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

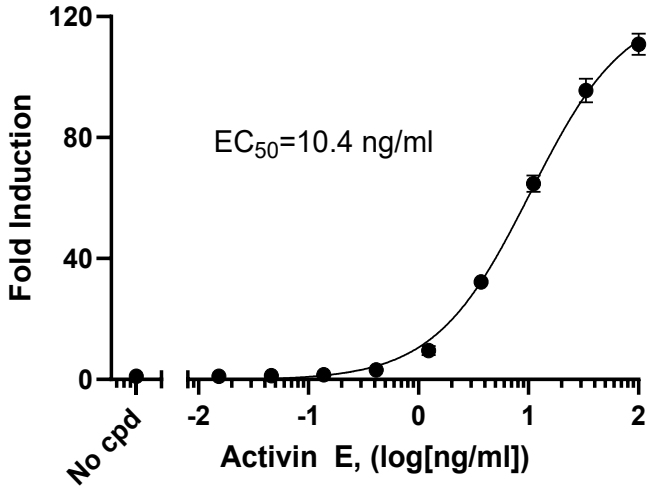

Figure 2. Dose-response curve of Activin E Responsive Luciferase Reporter HEK293 Cell Line to human Activin E.
Cells were treated with increasing concentrations of Activin E. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

B. Inhibition of Activin E-induced luciferase activity in Activin E Responsive Luciferase Reporter HEK293 Cell Line by an ALK inhibitor

1. Seed Activin E Responsive Luciferase Reporter HEK293 cells at a density of 30,000 cells/well in 100 µl of Thaw Medium 1, into a white clear-bottom 96-well cell culture plate. Leave a few empty wells to determine the background luminescence ("Cell-Free Control").
2. Incubate the cells at 37°C with 5% CO_2 overnight.
3. Prepare a three-fold serial dilution of inhibitor in Assay Medium (50 µl/well) at concentrations 2x higher than the desired final concentrations.
4. Remove the cell culture medium from the cells.
5. Add 50 µl of each dilution to the "Stimulated, Test Compound" wells.
6. Add 50 µl of Assay Medium to the "Unstimulated, No Compound" and "Stimulated, No Compound" wells.
7. Incubate the cells at 37°C in 5% CO_2 for 1-2 hours.
8. Prepare Activin E in Assay Medium at 2x the desired final concentration (50 µl/well).
9. Add 50 µl of Activin E to the "Stimulated, No Compound" and "Stimulated, Test Compound" wells.
10. Add 50 µl of Assay Medium to the "Unstimulated, No Compound" wells.

11. Add 100 µl of Assay Medium to “Cell-Free Control” wells.

12. Incubate at 37°C in 5% CO_2 for 5-6 hours.

13. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.

14. Incubate with gentle agitation at RT for ~15 minutes.

15. Measure luminescence using a luminometer.

Data Analysis

Subtract the average background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of “Stimulated, Test Compound” cells divided by the background-subtracted luminescence of “Stimulated, No Compound” cells, multiplied by 100.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \text{"Stimulated, Test Compound"}\ cells - avg.background}{Avg.Luminescence\ of\ "Stimulated, no\ Compound"\ cells" - avg.background}\right) x100$$

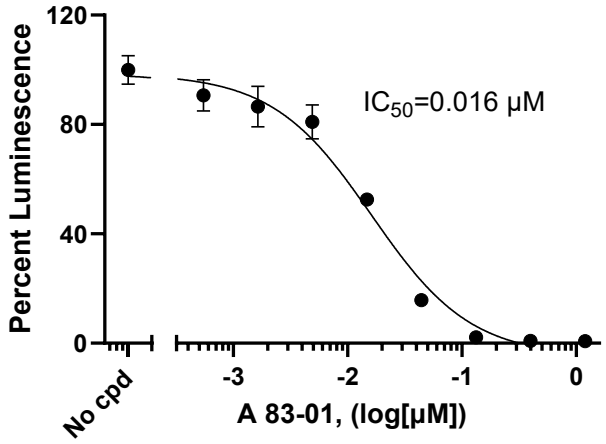

Figure 3. Inhibition of Activin E-induced reporter activity by A 83-01 in Activin E Responsive Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of A 83-01 (#84236) for 1 hour followed by incubation with Activin E for 5 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of Activin E luciferase reporter activity compared to the activity of cells without antagonist (set at 100%).

Data shown is representative.

References

Adam R., *et al*., 2023 *Proc. Natl. Acad. Sci. U.S.A.* 120 (32) e2309967120.
Carlsson L., *et al*., 2009 *Biochem Biophys Res Commun.* 382(2):309-14.
Guo T., *et al*., 2014 *eLife*. 3:e03245.
Ibáñez C.F., 2022 *FEBS J.* 289: 5776-5797.
Vestal K., *et al*., 2024 *Biochem J.* 481(7):547-564.

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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.

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