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Produktinformation

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

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The IL-11 (Interleukin-11) Responsive Luciferase Reporter HEK293 Line is a HEK293 cell line engineered to express both human IL-11Ra (IL-11 receptor alpha) (NM_001142784.3) and human IL-6ST (IL-6 cytokine family signal transducer, also known as gp130) (NM_002184.4) separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of STAT3 Luciferase Reporter HEK293 Cell Line (#79800-P), which express a firefly luciferase reporter driven by STAT3 response elements located upstream of the minimal TATA promoter. After activation by IL-11, the endogenous transcription factor STAT3 binds to the response elements, inducing transcription of the luciferase reporter gene.

This cell line has been validated to respond to IL-11.

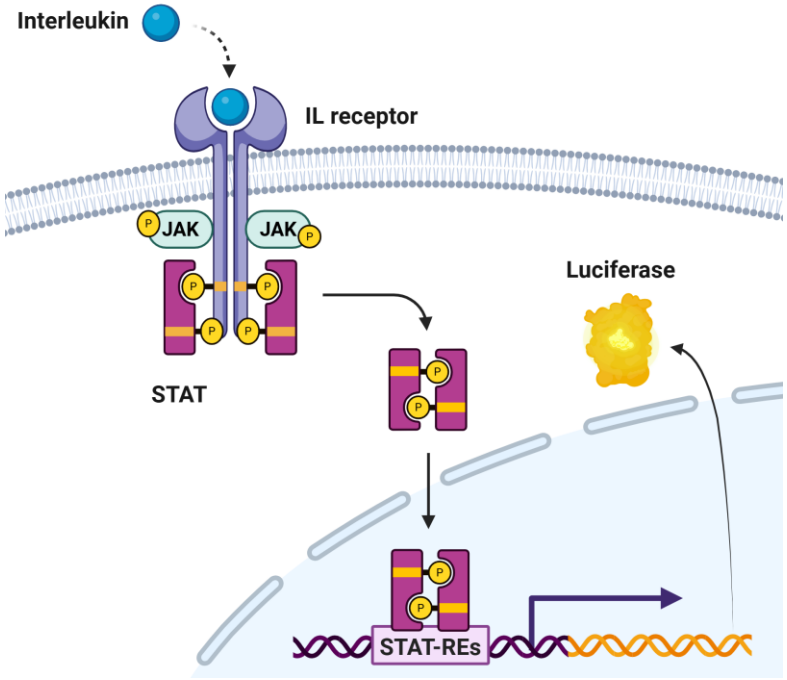

Figure 1: Illustration of the mechanism of action in the IL-11 Responsive Luciferase Reporter HEK293 Cell Line.

Background

Interleukin-11 (IL-11) is multifunctional cytokine that belongs to the IL-6 family of cytokines, which commonly activate glycoprotein 130 (gp130), a signal transducing receptor protein. Initially recognized for its role in hematopoiesis, further research has indicated that IL-11 may play an important role in the pathogenesis of fibro-inflammatory diseases. IL-11 is produced in tissues throughout the body with the primary cellular producer being stromal fibroblasts found in the lungs, liver, heart, and GI (gastrointestinal) tract. Signaling is initiated when IL-11 binds to its specific membrane bound receptor, IL-11 receptor alpha (IL-11Rα or IL-11Ra), followed by binding to its co-receptor gp130 (IL-6ST, IL-6 cytokine family signal transducer) and subsequent dimerization with another gp130 molecule. This results in the activation of Janus kinases (JAKs), predominately JAK1 and JAK2, prompting phosphorylation and activation of signal transducer and activator of transcription (STAT) molecules STAT1 and STAT3. While typically undetectable in healthy individuals, elevated expression of IL-11 has been identified in the tissues of patients with idiopathic pulmonary fibrosis, kidney fibrosis, and cardiac fibrosis. The development of therapeutics targeting IL-11 is an active area of research with clinical trials currently ongoing for the treatment of fibrotic diseases.

Application

- Screen for modulators of IL-11 mediated signaling pathways.

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 1U	BPS Bioscience #78548

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Human IL-11 Protein	BPS Bioscience #83545
White, clear-bottom cell culture plate, 96-well	Corning #3610
White, Tissue Culture treated plate, 384-well	PerkinElmer #6007680
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 1U (BPS Bioscience #78548):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 0.5 µg/ml Puromycin and 100 µg/ml Hygromycin B.

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 passage.

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

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 1U 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 1U.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10-1:20 once or twice per 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 1U 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 at least 10 vials at an early passage for future use.

Functional Validation

- The following assays are designed for 96-well (protocol A) and 384-well format (protocol B). 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 and B should include “Cell-Free Control”, “Unstimulated Control” and “Stimulated” conditions.

A. 96-Well Assay Format: Dose-response of IL-11 Responsive Luciferase Reporter HEK293 Cell Line to recombinant human IL-11.

1. Seed IL-11 Responsive Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate 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. Prepare a serial dilution of recombinant human IL-11 at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

4. Add 10 µl of diluted IL-11 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 expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

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

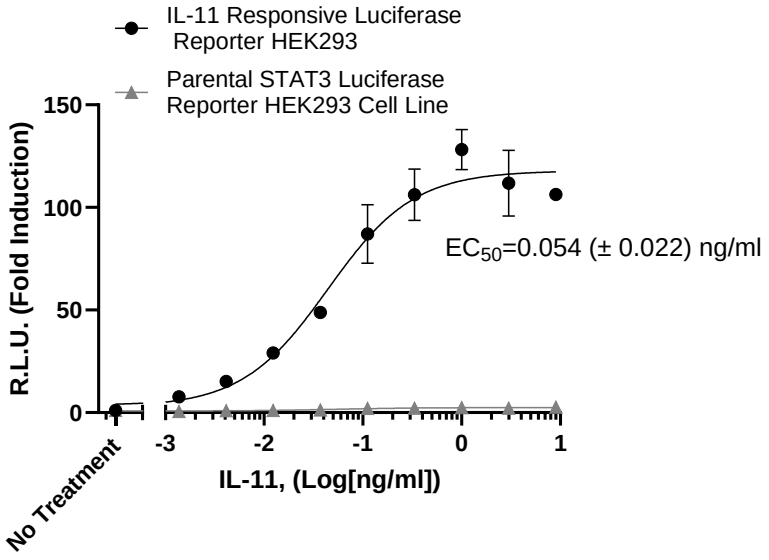

Figure 2. Dose response curve of IL-11 Responsive Luciferase Reporter HEK293 Cell Line and parental STAT3 Luciferase Reporter HEK293 Cell Line to recombinant human IL-11 in a 96-well assay format.
IL-11 Responsive Luciferase Reporter HEK293 cells STAT3 Luciferase Reporter HEK293 cells (#79800-P) were treated with increasing concentrations of IL-11 in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

B. 384-Well Assay Format: Dose-response of IL-11 Responsive Luciferase Reporter HEK293 Cell Line to recombinant human IL-11.

1. Seed IL-33 Responsive Luciferase Reporter Jurkat cells into a white 384-well plate at a density of 10,000 cells per well in 20 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. Prepare a serial dilution of IL-11 at concentrations 5-fold higher than the desired final concentrations in Assay Medium (5 µl/well).

4. Add 5 µl of diluted IL-11 to the "Stimulated Cells" wells.

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

6. Add 25 µl of Assay medium to the "Cell-Free Control" wells.

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

8. Add 25 µl of the ONE-Step™ Luciferase reagent to each well.

9. Rock gently at 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 expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

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

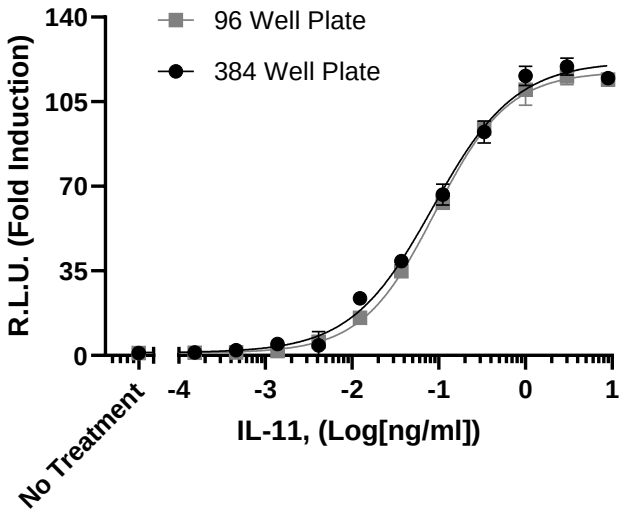

Figure 4. Dose-response curve of IL-11 Responsive Luciferase Reporter HEK293 Cell Line in response to recombinant human IL-11 in 96 and 384-well format.
IL-11 Responsive Luciferase Reporter HEK293 cells were treated with increasing concentrations of IL-11 in a 96-well or 384-well plate format. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

Data is representative.

References

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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*
STAT3 Luciferase Reporter HEK293 Cell Line	79800-P	2 vials
IL-23 Responsive STAT3 Luciferase Reporter HEK293 Cell Line	82591	2 vials
IL-31 Responsive Luciferase Reporter HEK293 Cell Line	82799	2 vials
IL-6 Responsive Luciferase Reporter HEK293 Cell Line	82235	2 vials

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