

## Description

The IL-18 Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express both human IL-18R1 (interleukin-18 receptor 1) (NM\_003855.5) and human IL18RAP (Interleukin 18 receptor accessory protein) (NM\_003853.3) separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of NF- $\kappa$ B Luciferase Reporter HEK293 Cell Line (#60650), which expresses a firefly luciferase reporter driven by NF $\kappa$ B (nuclear factor Kappa B) response elements located upstream of the minimal TATA promoter. After activation by IL-18, the endogenous transcription factor NF- $\kappa$ B binds to the response elements, inducing transcription of the luciferase reporter.

This cell line has been validated to respond to IL-18 and to IL-18 targeting antibodies.

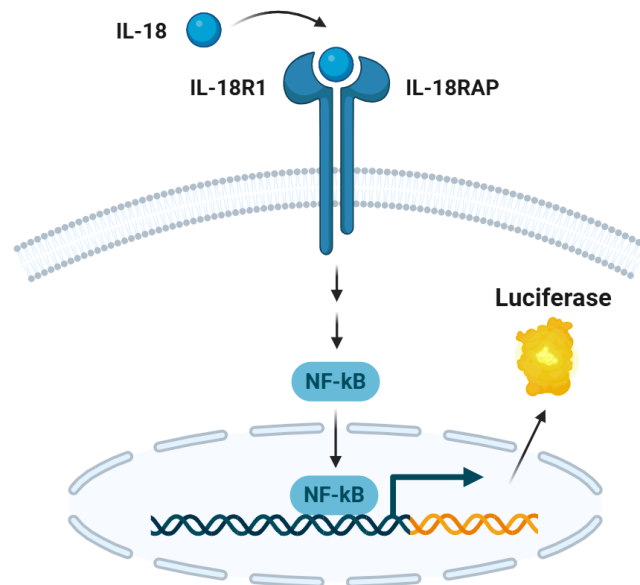


Figure 1: Illustration of the mechanism of reporter activation in IL-18 Luciferase Reporter HEK293 Cell Line.

## Background

Interleukin 18 is a pro-inflammatory cytokine belonging to the IL-1 superfamily of cytokines and plays an important role as a modulator of both innate and adaptive immunity. The inactive precursor form of IL-18 (pro-IL-18) is constitutively expressed in the cytoplasm of both hematopoietic and non-hematopoietic cells including macrophages, dendritic cells, keratinocytes, and epithelial cells. IL-18 signaling is initiated via inflammasome mediated activation of caspase-1 which cleaves the precursor to produce active IL-18. Mature IL-18 binds to the IL-18 alpha chain (IL-18R $\alpha$ ) and IL-18 receptor beta chain (IL-18R $\beta$ , also known as IL-18RAP) to form a high affinity signaling complex that activates downstream signaling pathways including NF- $\kappa$ B (nuclear factor Kappa B) and MAPK (mitogen activated protein kinase). In the presence of IL-12 or IL-15, IL-18 drives Th1 responses by signaling to T cells and NK cells to induce IFN $\gamma$  (interferon gamma) production, which is essential for immune responses to infections. Additionally, IL-18 upregulates the expression of genes critical for cell survival/differentiation such as TNF (tumor necrosis factor) and FasL (Fas ligand) for cell death. Therapeutic strategies designed to neutralize excess IL-18 is a growing field of research showing promising results for the treatment of autoinflammatory diseases. GSK-1070806 (Aletektug) is a monoclonal antibody that neutralizes IL-18 to treat atopic dermatitis (AD). Additional therapies include Tadekinig alfa, which is a recombinant IL-18 binding protein that inhibits free IL-18 and was developed for the treatment of NLRC4-MAS (NLR family caspase activation and recruitment domain-containing 4 Macrophage Activation Syndrome) and XIAP (X-linked inhibitor of apoptosis protein) deficiency.

**Application**

- Screen and characterize modulators of IL-18 signaling.

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ 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 19B	BPS Bioscience #83761

*Materials Required for Cellular Assay*

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
IL-18 (interleukin 18) Recombinant (Human)	BPS Bioscience #84152
GSK-1070806	BPS Bioscience #84153
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  $-80^{\circ}\text{C}$  freezer for long term storage. Contact technical support at [support@bpsbioscience.com](mailto: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<sub>2</sub>. 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 19B (BPS Bioscience #83761):*

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 50 µg/ml Hygromycin and 0.5 µg/ml 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<sub>2</sub> 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<sub>2</sub> 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 19B.

#### *Cell Passage*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 19B 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 19B.
4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 once or twice a week.

### Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup>, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 19B 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<sup>6</sup> 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 IL-18 Responsive Luciferase Reporter HEK293 Cell Line to IL-18 agonist

1. Seed IL-18 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<sub>2</sub> incubator overnight.
3. Prepare a serial dilution of agonist at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

4. Add 10  $\mu$ l of each dilution to the “Stimulated” wells.
5. Add 10  $\mu$ l of Assay Medium to the “Unstimulated Control” (negative control) wells.
6. Add 100  $\mu$ l of Assay Medium to the “Cell-Free Control” wells (for determining background luminescence).
7. Incubate the plate at 37°C in a CO<sub>2</sub> incubator for 5-6 hours.
8. Add 100  $\mu$ l 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.

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

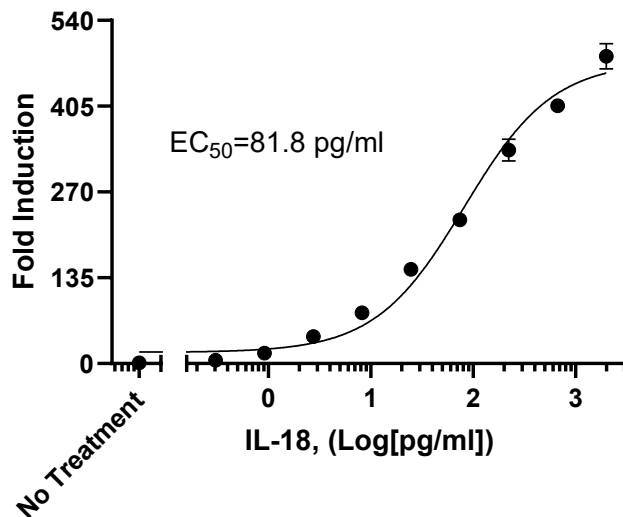


Figure 2. Dose response curve of IL-18 Responsive Luciferase Reporter HEK293 Cell Line to human IL-18.

Cells were treated with increasing concentrations of IL-18 for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

**B. Inhibition of IL-18 stimulation by anti-IL-18 antibodies in IL-18 Responsive Luciferase Reporter HEK293 Cell****Line**

1. Seed IL-18 Responsive Luciferase Reporter HEK293 cells at a density of 30,000 cells/well in 80 µl of Assay Medium, 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<sub>2</sub> overnight.
3. Prepare a solution of human IL-18 at 10x the final concentration in Assay Medium (10 µl/well).
4. Prepare a 3-fold serial dilution of anti-IL-18 antibody at 10x the desired final concentration in Assay Medium (10 µl/well).
5. Combine equal volumes of IL-18 and anti-IL-18 antibody prepared in step 3 and 4, and mix.
6. Add 20 µl of antibody mixture to the "Stimulated, Test Compound" wells.
7. Add 10 µl of human IL-18 and 10 µl of Assay Medium to the "Stimulated, No Compound" wells.
8. Add 20 µl of Assay Medium to "Unstimulated, No Compound" wells.
9. Add 100 µl of Assay Medium to "Cell-Free Control" wells.
10. Incubate at 37°C in 5% CO<sub>2</sub> for 5-6 hours.
11. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.
12. Incubate with gentle agitation at RT for ~15 minutes.
13. Measure luminescence using a luminometer.

**Data Analysis**

Subtract the background luminescence from the luminescence reading of all the wells. The Percent Activity of IL-18 luciferase reporter expression is the background-subtracted luminescence of "Stimulated, Test Compound" cells divided by the background-subtracted luminescence of the "Stimulated, No Compound" treated wells, multiplied by 100.

*% Activity*

$$= \left( \frac{\text{Luminescence of "Stimulated, Test compound" treated cells} - \text{avg. background}}{\text{Avg. Luminescence of "Stimulated, No compound" treated cells} - \text{avg. background}} \right) \times 100$$

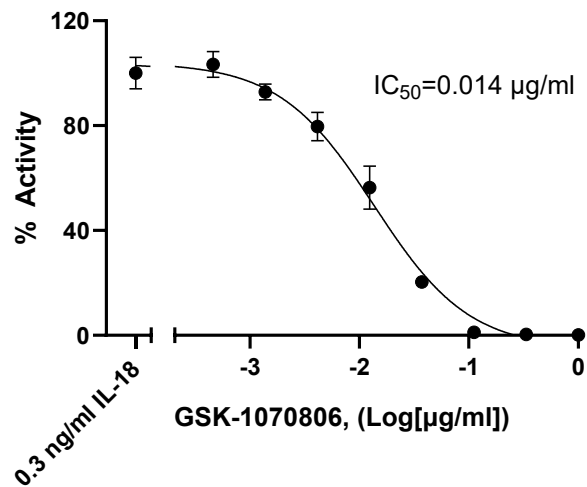


Figure 3. Dose-dependent response of IL-18 Responsive Luciferase Reporter HEK293 Cell Line to GSK-1070806.

Cells were incubated with increasing concentrations of GSK-1070806 and 0.3 ng/ml of IL-18 for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. The results are shown as percentage of NF-κB luciferase reporter activity compared to the activity of cells without antagonist (set at 100%).

Data shown is representative.

#### References

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 Gracie J., *et al.*, 2003 *J Leukoc Biol.* 73(2):213-24  
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 Landy E., *et al.*, 2024 *Nat Rev Rheumatol.* 20(1):33-47  
 Kaplanski G., 2018 *Immunol Rev.* 281(1):138-153

#### License Disclosure

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#### Troubleshooting Guide

Visit [bpsbioscience.com/cell-line-faq](https://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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