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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

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

IL-6 Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line designed to monitor the IL-6 (interleukin 6)/IL-6 receptor interaction. These cells express endogenous IL-6 receptor (IL-6R and gp130) and were engineered to express firefly luciferase under the control of a STAT response element (*Figure 1*).

This cell line has been validated to respond to human interleukin-6 (IL-6), IL-27, and further functional validation experiments showed that IL-6-induced luciferase activity was decreased by the JAK inhibitor CP 690,550 and by an anti-IL-6R neutralizing antibody.

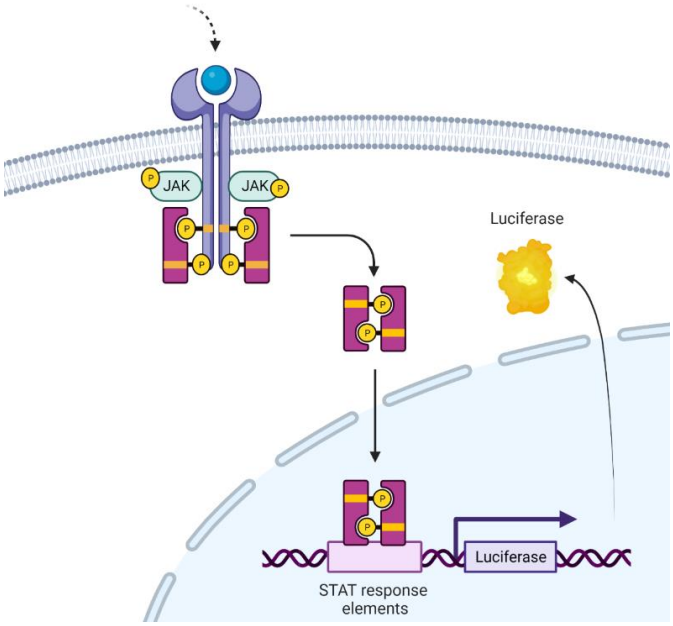

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Figure 1: Illustration of the mechanism behind reporter expression in IL-6 Responsive Luciferase Reporter HEK293 Cell Line.

Background

IL-6 (interleukin-6), instantly produced at the site of inflammation, plays a critical role in host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. IL-6 exerts stimulatory effects on T- and B-cells, by acting on the STAT3 (signal transducer and activator of transcription 3) signaling pathway. Dysregulated IL-6 expression shows a pathological effect on chronic inflammation, autoimmunity and cancer. Il-6 expression is regulated at multiple levels, with transcription being induced by several types of stimuli, such as TLR (Toll-like receptor) ligands, TNFα (tumor necrosis factor alpha) and ROS (reactive oxygen species). IL-6 is also involved in cell senescence and can trigger senescence-induced inflammation and age-related diseases. The use of the anti-IL-6R antibody tocilizumab has resulted in positive outcomes in the treatment of RA (rheumatoid arthritis) and Castleman's disease. Hence, the development of molecules able to target the IL-6 and IL-6 signaling pathway is an active area of research and can be an effective strategy for prevention and treatment of chronic inflammatory diseases and cancer.

Application

- Monitor IL-6 activity.
- Study the effect of compounds on IL-6/IL-6R mediated signaling pathways.
- Screen for anti-IL-6 or anti-IL-6R 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)

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 1N	BPS Bioscience #79801

Materials Required for Cellular Assays

Name	Ordering Information
Human IL-6	R&D Systems #206-IL
Human IL-27	R&D Systems #2526-IL
JAK inhibitor CP 690,550	Cayman #11598
Anti-IL-6R Antibody	R&D Systems #MAB227
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
96-well tissue culture white, clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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*. 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℃ 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 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, and 0.5 µg/ml of Puromycin

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)

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.

   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 1N.

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 1N 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 1N.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 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 1N 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 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.

Validation Data

- The following assay was designed for a 96-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.

A. Dose response of IL-6 Responsive Luciferase Reporter HEK293 Cell Line to Human IL-6

The assay should include "Stimulated Cells", "Background Control" and "Unstimulated Control" conditions.

1. Seed IL-6 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. Keep wells without cells as "Background Control".

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a serial cytokine dilution (we recommend a 3-fold increment serial dilution) in Assay Medium at the desired final concentrations (100 µl/well).

4. Remove the medium from the cells.

5. Add 100 µl of each cytokine dilution to the "Stimulated Cells" wells.

6. Add 100 µl of Assay Medium to the "Unstimulated Control" (for measuring basal level of STAT reporter activity).

7. Add 100 µl of Assay Medium to "Background Control" (cell free wells).

8. Incubate at 37°C with 5% CO_2 for ~18 hours.

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

10. Incubate with gentle agitation at Room Temperature (RT) for ~15 minutes.

11. Measure luminescence using a luminometer.

12. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The fold induction of IL-6 responsive luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

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

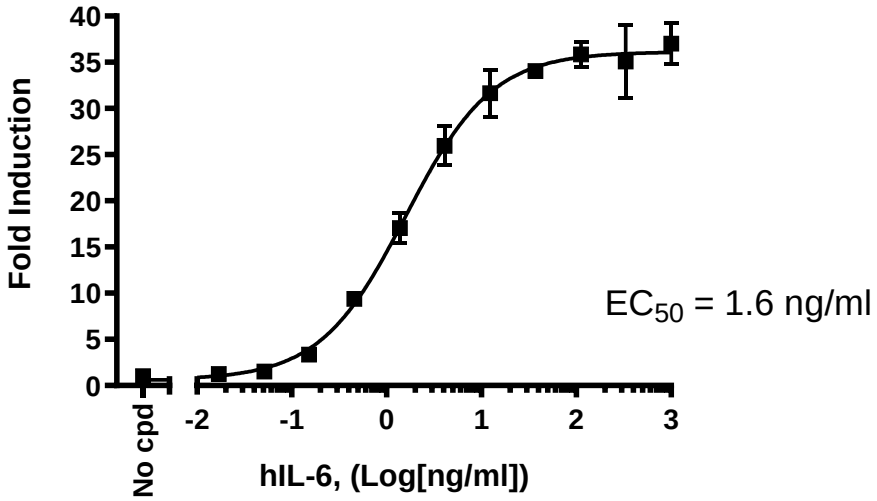

Figure 2. Dose response curve of IL-6 Responsive Luciferase Reporter HEK293 Cell Line to hIL-6. IL-6 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human IL-6 overnight and luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression (compared to unstimulated cells).

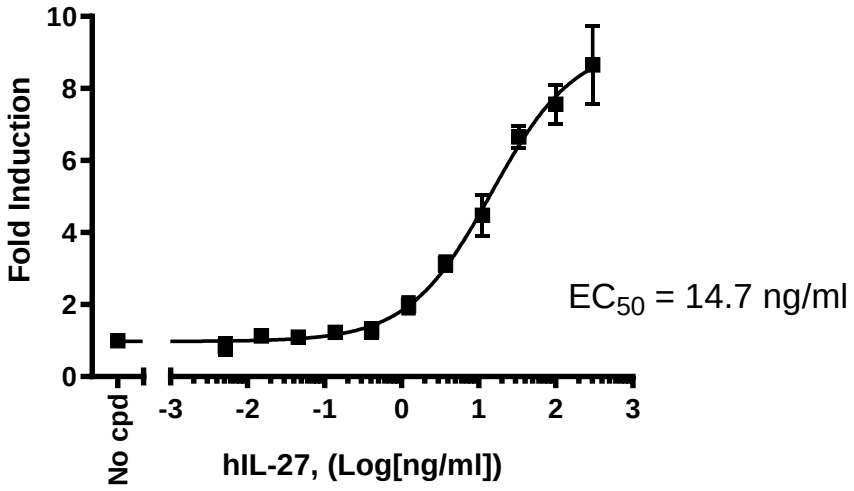

Figure 3. Dose response curve of IL-6 Responsive Luciferase Reporter HEK293 Cell Line to hIL-27. IL-6 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human IL-27 overnight and luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression (compared to unstimulated cells).

B. Inhibition of hIL-6 responsive activity by a JAK inhibitor

- The assay should include "Stimulated, No Inhibitor", "Unstimulated, No Inhibitor", "Background Luminescence" and "Test Inhibitor".
- Inhibitor testing should be performed on cells stimulated with an agonist.
- If the test compound is dissolved in DMSO, keep the final concentration of DMSO at a maximum of 0.1%.

1. Seed IL-6 Responsive Luciferase Reporter HEK293 cells at a density of 30,000 cells/well in 100 µl of Thaw Medium 1 into white clear-bottom 96-well cell culture plate. Keep wells without cells as "Background Control".

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare Test Inhibitor (50 µl/well): for a titration prepare serial dilutions at concentrations 2-fold higher than the desired final concentrations. The final volume of the reaction is 100 µl.

   3.1 If the test inhibitor is soluble in water, make a dilution in Assay Medium at a concentration 2-fold higher than the final desired concentration.

   For the positive and negative controls, use Assay Medium (Diluent Solution).

   OR

   3.2 If the Test Inhibitor is soluble in DMSO: Prepare the test inhibitor at 1000-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 500-fold in Assay Medium to prepare the highest concentration of the 2-fold intermediate dilutions. The concentration of DMSO is now 0.2%.

Prepare serial dilutions of the Test Inhibitor at 2-fold the desired final concentrations using 0.2% DMSO in Assay Medium to keep the concentration of DMSO constant.

For positive and negative controls, prepare 0.2% DMSO in Assay Medium so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 0.1%.

Note: Results shown in Figure 4 were obtained by preparing a stock solution of CP 690,550 at 10 mM in DMSO, which was then diluted to 20 µM in Assay Medium. The serial dilution was prepared at concentrations 2-fold higher than the final desired concentrations in Assay Medium containing 0.2% DMSO (Diluent Solution), to keep the concentration of DMSO constant.

4. Remove the cell culture medium from the cells.
5. Add 50 µl of diluted inhibitor to the "Test Inhibitor" wells.
6. Add 50 µl of Diluent Solution to the "Stimulated, No Inhibitor", and "Unstimulated, No Inhibitor" wells.
7. Incubate the cells at 37°C with 5% CO_2 for 1-2 hours.
8. Prepare IL-6 in Assay Medium at a concentration of 20 ng/ml (the final concentration will be 10 ng/ml).
9. Add 50 µl of diluted hIL-6 to the "Stimulated, No Inhibitor" and "Test Inhibitor" wells.
10. Add 50 µl of Assay Medium to the "Unstimulated, No Inhibitor" wells (for determining STAT basal activity).
11. Add 100 µl of Assay Medium to the "Background Control" wells.
12. Incubate at 37°C with 5% CO_2 for ~18 hours.
13. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.
14. Incubate with gentle agitation at RT for ~15 to 30 minutes.
15. Measure luminescence using a luminometer.
16. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of inhibitor-treated cells divided by the background-subtracted luminescence of untreated control cells ("Unstimulated, No Inhibitor"), multiplied by 100. IL-6-stimulated cells in the absence of JAK inhibitor are set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ inhibitor\ treated\ cells - background}{luminescence\ of\ untreated\ cells - background}\right) x100$$

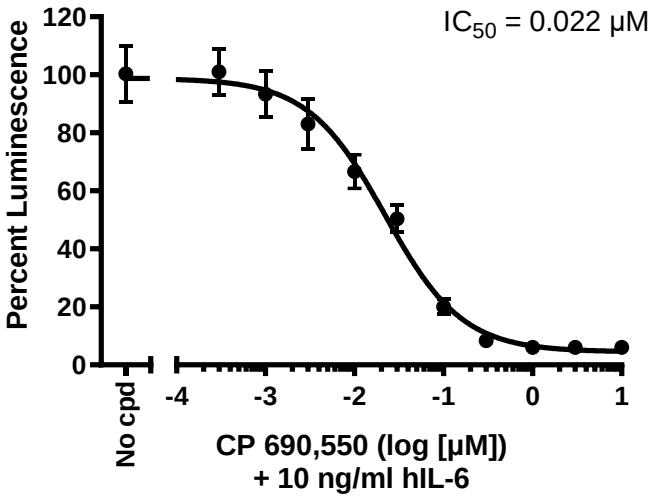

Figure 4. Inhibition of hIL-6-induced reporter activity by the JAK Inhibitor CP 690,550 in IL-6 Responsive Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of JAK inhibitor for 2 hours, followed by incubation with IL-6 (10 ng/ml) overnight. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage luminescence of IL-6 responsive luciferase reporter activity (compared to cells stimulated by IL-6, without inhibitor).

C. Inhibition of hIL-6 responsive luciferase activity by an anti-IL-6R antibody

- The assay should include "Stimulated, No Antibody", "Unstimulated, No Antibody", "Background Luminescence" and "Neutralizing Antibody".
- Blocking antibody testing should be performed on cells stimulated with an agonist.

1. Seed IL-6 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. Keep wells without cells as "Background Control" (for determining the background luminescence).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a three-fold serial dilution of anti-IL-6R antibody in Assay Medium (50 µl/well) at concentrations 2-fold higher than the desired final concentrations.

4. Remove the cell culture medium from the cells.

5. Add 50 µl of each dilution to the "Neutralizing Antibody" wells.

6. Add 50 µl of Assay Medium to the "Stimulated, No Antibody" and "Unstimulated, No Antibody" wells.

7. Incubate the cells at 37°C in 5% CO2 for 1-2 hours.

8. Prepare hIL-6 in Assay Medium at a concentration of 20 ng/ml (the final concentration will be 10 ng/ml).

9. Add 50 µl of hIL-6 to the "Neutralizing Antibody" and "Stimulated, No Antibody" wells.

10. Add 50 µl of Assay Medium to the "Unstimulated, No Antibody" (for determining STAT basal activity) wells.
11. Add 100 µl of Assay Medium to "Background Control" wells.
12. Incubate at 37°C in 5% CO_2 for ~18 hours.
13. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.
14. Incubate with gentle agitation at RT for ~15 to 30 minutes.
15. Measure luminescence using a luminometer.
16. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of inhibitor-treated cells divided by the background-subtracted luminescence of untreated control cells ("Unstimulated, No Antibody"), multiplied by 100. IL-6-stimulated cells in the absence of neutralizing antibody are set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ inhibitor\ treated\ cells - background}{luminescence\ of\ untreated\ cells - background}\right) x100$$

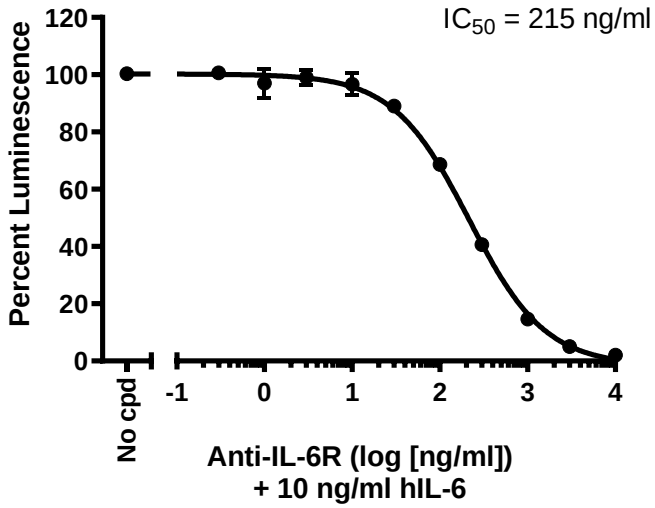

Figure 5. Inhibition of hIL-6-induced reporter activity by an anti-IL-6R antibody in IL-6 Responsive Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of anti-IL6 neutralizing antibody for 2 hours and incubated with IL-6 (10 ng/ml) overnight. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of IL-6 responsive luciferase reporter activity (compared to cells stimulated by IL-6, without antibody).

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

Tanaka T., *et al.,* 2014, *Cold Spring Harb Perspect Biol*. 6(10):a016295.
Choy EH., *et al.,* 2020, *Nature Rev Rheumatol*. 16:335-345.
Hirano T., 2021 *International Immunology* 33(3):127-148.

Related Products

Products	*Catalog #*	*Size*
STAT3 Reporter Kit	79730	500 reactions
STAT3 Luciferase Reporter Lentivirus	79744	2 x 500 µl
Human IL-6	90196-B	20 µl
STAT3, GST-Tag	75003	20 µg
STAT5 Luciferase Reporter Ba/F3 Cell line	79772	2 vials
STAT3 Luciferase Reporter THP-1 Cell Line	78498	2 vials

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