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Produktinformation

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

IL-31 Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses the human IL-31 receptor complex, composed of human IL-31 receptor alpha (hIL-31RA [NM_139017.7]) and human oncostatin M receptor beta (hOSMR [NM_003999.3]) separated by a self-cleaving P2A peptide. The cell line was generated by lentiviral transduction of STAT3 Luciferase Reporter HEK293 Cell Line (#79800-P), which express firefly luciferase driven by STAT3 response elements located upstream of a minimal TATA promoter. IL-31 activity can be monitored by measuring luciferase activity.

The functionality of this cell line was validated as responding to human IL-31, and by showing that IL-31-induced luciferase activity can be inhibited by anti-IL-31 neutralizing antibodies.

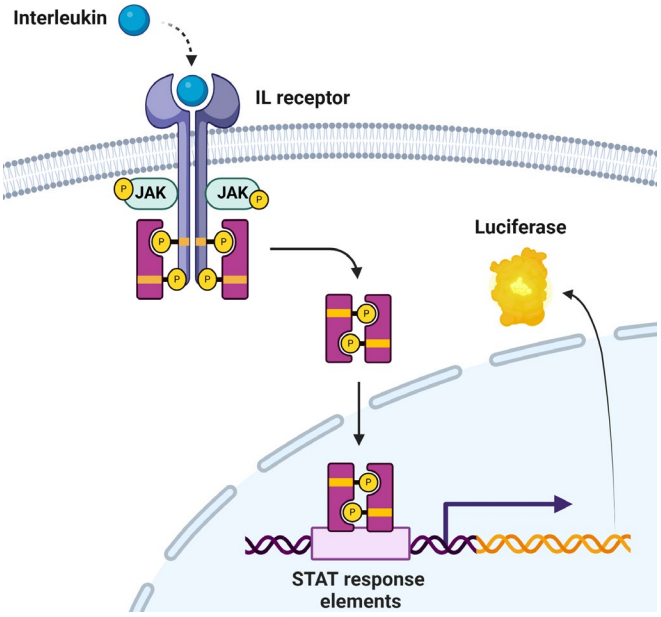

Figure 1: Illustration of the IL-31 Responsive Luciferase Reporter HEK293 Cell Line.
IL-31 initially binds to IL-31RA and then to OSMR to form a high affinity receptor complex. This binding leads to phosphorylation of STAT3 by JAK1/2. After phosphorylation, STAT3 is translocated to the nucleus where it binds to STAT response elements and induces the transcription of firefly luciferase.

Background

IL-31 (interleukin 31) is a four-helix bundle proinflammatory cytokine preferentially produced by T helper type 2 (T_H2) cells. IL-31 regulates cell differentiation, cell proliferation, and immune responses, serving as a neuroimmune link between T_H2 cells and sensory neurons in generating a T cell-mediated inflammatory itch. The IL-31 receptor complex, composed of IL-31RA and OSMR (human oncostatin M receptor beta), is expressed by various epithelial and immune cells as well as dorsal root ganglia sensory neurons. IL-31 interacts with the IL-31 receptor complex, activating Janus kinases JAK1 and JAK2. This activation leads to the phosphorylation of signal transducer and activator of transcription (STAT) molecules, mainly STAT3, which upregulate the genes responsible for the IL-31 induced itch sensation. Often referred to as the itchy cytokine, increased expression of IL-31 or its receptor IL-31RA is correlated with alopecia, skin lesions, airway hypersensitivity, and particularly pruritic disorders such as atopic dermatitis. IL-31 and other T_H2 related cytokines (including IL-4, IL-13 and TSLP (thymic stromal lymphopoietin) play an important role in the pathogenesis of a variety of inflammatory and allergic diseases and have become popular for therapeutic development. The IL-31RA targeting antibody Nemolizumab has been FDA-approved for the treatment of atopic dermatitis.

Application

- Screen and characterize modulators of IL-31 activity.
- Screen anti-IL-31 and anti-IL-31RA 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 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
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Human Interleukin-31 Recombinant	BPS Bioscience #90190-B
Anti-IL-31RA Neutralizing Antibody	BPS Bioscience #102378
Nemolizumab	BPS Bioscience #82880
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom 96-well tissue culture plate	Corning #3610
White, opaque 384-well tissue culture plate	PerkinElmer #6007680
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 15 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

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 1:6 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 Data

- The following assays are designed for 96-well or 384-well format, as specified. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- Assay A and B measures the effect of a compound on reporter activation.
- Assay C measures effect of an antagonist compound against agonist activation.
- All conditions should be performed in triplicate.
- Assay A and B should include “Unstimulated”, “Cell-Free" (Background luminescence) and “Stimulated” conditions.
- Assay C should include “Stimulated’, “Antagonist”, “Stimulated, No Antagonist,” “Unstimulated, No Antagonist” and “Cell-Free” control conditions.

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

A. Dose response to IL-31 in IL-31 Responsive Luciferase Reporter HEK293 Cell Line in 96-well format

1. Seed cells at a density of 30,000 cells/well in 90 µl of Assay Medium into a white, clear-bottom 96-well plate. Leave a few empty wells as “Cell-Free” control (Background luminescence).

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

3. Prepare a 3-fold serial dilution of human IL-31 at 10-fold the desired final concentrations in Assay Medium (10 µl/ well).

4. Add 10 µl of the agonist dilutions to each "Stimulated" well.
5. Add 10 µl of Assay Medium to the "Unstimulated" wells.
6. Add 100 µl of Assay Medium to the "Cell-Free" control wells (background luminescence).
7. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.
8. Add 100 µl of ONE-Step™ reagent to each well.
9. Incubate with agitation at Room Temperature (RT) for ~15 minutes.
10. Measure luminescence using a luminometer.
11. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of STAT3 luciferase reporter expression is the background-subtracted luminescence of stimulated wells divided by the background-subtracted luminescence of unstimulated control wells.

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

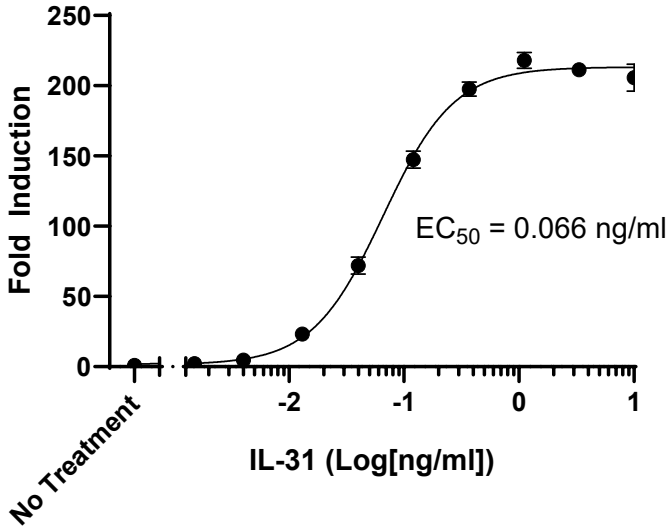

Figure 2: Reporter activation observed in IL-31 Responsive Luciferase Reporter HEK293 Cell Line in response to human IL-31.
IL-31 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human IL-31 for 5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of STAT3 luciferase reporter expression in relation to the activity of cells without agonist.

B. Dose response to IL-31 in IL-31 Responsive Luciferase Reporter HEK293 Cell Line in 384-well format

1. Seed cells at a density of 5,000 cells/well in 30 µl of Assay Medium into a white walled 384-well plate. Leave a few empty wells as "Cell-Free" control (Background luminescence).
2. Incubate the plate at 37°C with 5% CO_2 overnight.
3. Prepare a 3-fold serial dilution of human IL-31 at 4-fold the desired final concentrations in Assay Medium (10 µl/ well).
4. Add 10 µl of the agonist dilutions to each "Stimulated" well.
5. Add 10 µl of Assay Medium to the "Unstimulated" wells.
6. Add 40 µl of Assay Medium to the "Cell-Free" control wells (background luminescence).
7. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.
8. Add 40 µl of ONE-Step™ reagent to each well.
9. Incubate with agitation at RT for ~15 minutes.
10. Measure luminescence using a luminometer.
11. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of STAT3 luciferase reporter expression is the background-subtracted luminescence of stimulated wells divided by the background-subtracted luminescence of unstimulated control wells.

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

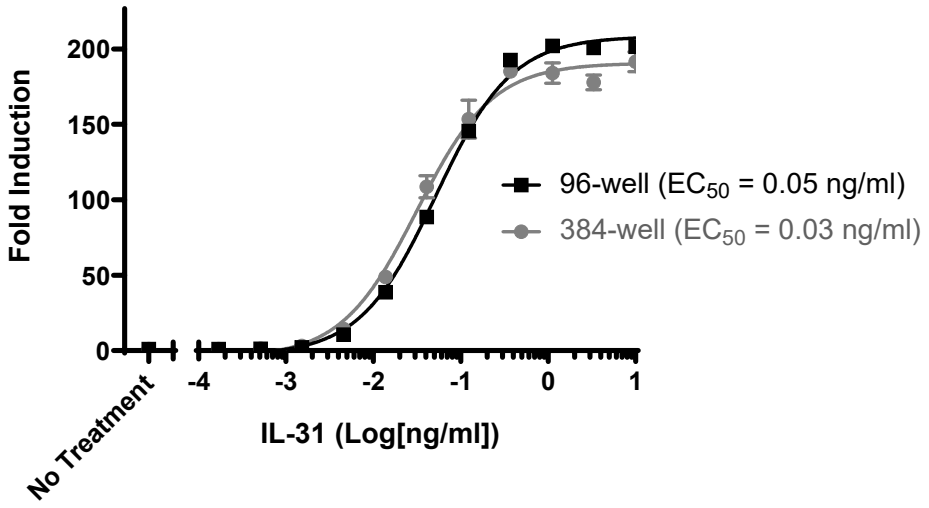

Figure 3: Reporter activation observed in IL-31 Responsive Luciferase Reporter HEK293 Cell Line in response to human IL-31 in 96-well and 384-well plates.
IL-31 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human IL-31 for 5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of STAT3 luciferase reporter expression in relation to the activity of cells without agonist.

C. Inhibition of human IL-31-induced STAT3 activity by anti-IL-31RA antibody in IL-31 Responsive Luciferase Reporter HEK293 Cell Line.

1. Seed cells at a density of 30,000 cells/well in 80 µl of Assay Medium into a white, clear-bottom white 96-well plate. Leave a few empty wells as "Cell-Free" control (Background luminescence).
2. Incubate the plate at 37°C with 5% CO_2 overnight.
3. Prepare a 3-fold serial dilution of anti-IL-31RA at 10-fold the desired final concentrations in Assay Medium (10 µl/well).
4. Add 10 µl of the antibody dilutions to each "Antagonist" well.
5. Add 10 µl of Assay Medium to the "Stimulated, No Antagonist," "Unstimulated, No Antagonist" wells.
6. Add 100 µl of Assay Medium to the "Cell-Free" control wells (background luminescence).
7. Incubate the plate at 37°C with 5% CO_2 for 30 minutes.
8. Prepare human IL-31 in Assay Medium at a concentration of 10 ng/ml (the final concentration per well will be 1 ng/ml) (10 µl/well).
9. Add 10 µl to each "Stimulated" well.

10. Add 10 µl of Assay Medium to “Unstimulated, No Antagonist” wells.

11. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.

12. Add 100 µl of ONE-Step™ reagent to each well.

13. Incubate with agitation at RT for ~15 minutes.

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence is the background-subtracted luminescence of antagonist treated cells divided by the background-subtracted luminescence of agonist activated control cells (“Stimulated, No Antagonist”) multiplied by 100. IL-31 stimulated cells in the absence of antagonist was set at 100%.

$$Percent\ Luminescence = \frac{Luminescence\ of\ Antagonist\ treated\ cells - avg.background}{Avg.Luminescence\ of\ "Stimulated,No\ Antagonist"\ cells - avg.background} \text{ x } 100$$

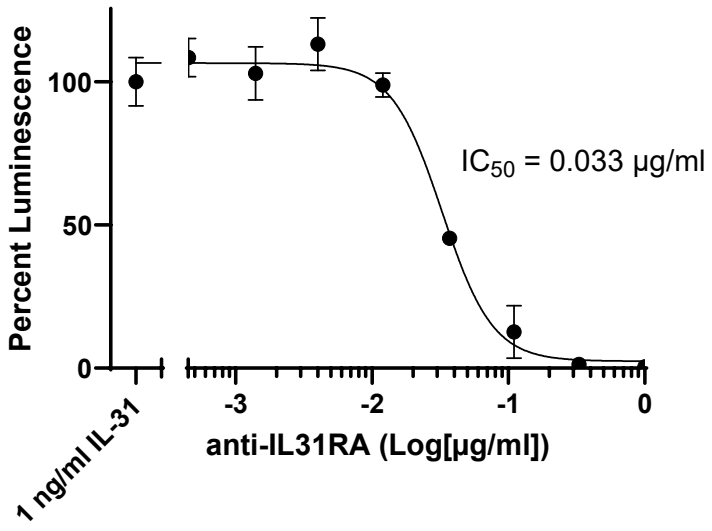

Figure 4: Inhibition of human IL-31-induced reporter activity by Anti-IL-31RA Neutralizing Antibody in IL-31 Responsive Luciferase Reporter HEK293 Cell Line.

IL-31 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of Anti-IL-31RA Neutralizing Antibody for 30 minutes, followed by treatment with human IL-31 (1 ng/ml) for 5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percentage of STAT3 luciferase reporter activity compared to the activity of cells without antagonist.

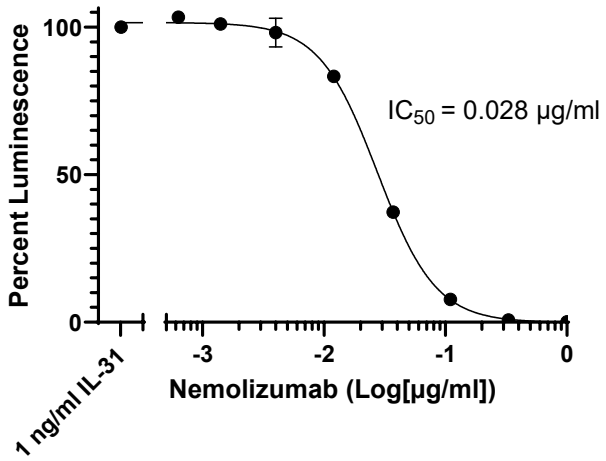

Figure 5: Inhibition of human IL-31-induced reporter activity by Nemolizumab in IL-31 Responsive Luciferase Reporter HEK293 Cell Line.

IL-31 Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of Nemolizumab for 30 minutes, followed by treatment with human IL-31 (1 ng/ml) for 5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percentage of STAT3 luciferase reporter activity compared to the activity of cells without antagonist.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

Human IL-31RA sequence (accession number NM_139017.7)

MCIRQLKFFTTACVCECPQNILSPQPSCVNLGMMWTWALWMLPSLCKFSLAALPAKPENISCVYYYRKNLTCTWSPGKETSYTQ
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FWCYNISVYPMLHDKVGEPYSIQAYAKEGVPSEGPETKVENIGVKTVTITWKEIPKSERKGIICNYTIFYQAEGGKGFSKTVNSSILQ
YGLESLKRKTSYIVQVMASTSAGGTNGTSINFKTLSFSVFEIILITSLIGGGLLILIILTVAYGLKKPNKLTHLCWPTVPNPAESSIATWH
GDDFKDKLNLKESDDSVNTEDRILKPCSTPSDKLVIDKLVVNFGNVLQEIFTDEARTGQENNLGGEKNGYVTCPFRPDCPLGKSFE
ELPVSPEIPPRKSQYLRSRMPEGTRPEAKEQLLFSGQSLVPDHLCEEGAPNPYLKNSVTAREFLVSEKLPEHTKGEV

Human OSMR sequence (accession number NM_003999.3)

MALFAVFQTTFFLTLLSLRTYQSEVLAERLPLTPVSLKVSTNSTRQSLHLQWTVHNLPYHQELKMVFQIQISRIETSNVIWVGNYST
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NIQNNVSCYLEGKQIHGEQLDPHVTAFNLNSVPFIRNKGTNIYCEASQGNVSEGMKGIVLFVSKVLEEPKDFSCETEDFKTLHCTW
DPGTDTALGWSKQPSQSYTLFESFSGEKKLCTHKNWCNWQITQDSQETYNFTLIAENYLRKRSVNILFNLTHRVYLMNPFSVNFE
NVNATNAIMTWKVHSIRNNFTYLCQIELHGEGKMMQYNVSIKVNGEYFLSELEPATEYMARVRCADASHFWKWSEWSGQNF
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VGASPASVIVISADPENKEVEEERIAGTEGGFSLSWKPQPGDVIGYVVDWCDHTQDVLGDFQWKNVGPNTTSTVISTDAFRPGV
RYDFRIYGLSTKRIACLLEKKTGYSQELAPSDNPHVLVDTLTSHSFTLSWKDYSTESQPGFIQGYHVYLKSKARQCHPRFEKAVLSDG

SECCKYKIDNPEEKALIVDNLKPESFYEFFITPFTSAGEGPSATFTKVTTPDEHSSMLIHILLPMVFCVLLIMVMCYLKSQWIKETCYP
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QMAVSLRLALPPPTENSSLSSITLLDPGEHYC

References

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Takahashi S., *et al.,* 2023 *Cell Rep.* 42(12): 113433

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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.

Related Products

Products	*Catalog #*	*Size*
STAT3 Luciferase Reporter HEK293 Cell Line	79800-P	2 vials
Human Interleukin-31 Recombinant	90190-B	10 µg
IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line	78941	2 vials
IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line	82731	2 vials
IL-33 Responsive Luciferase Reporter Jurkat Cell Line	82800	2 vials
TSLP Responsive Luciferase Reporter Ba/F3 Cell Line	82500	2 vials

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