Description

IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line is an HEK293 cell line engineered to express firefly luciferase under the control of interferon-γ-activated sequence of the interferon regulatory factor 1 (GAS-IRF1) promoter. It also expresses human STAT4 (signal transducer and activator of transcription 4) (NM_001243835.1), and the human IL-12 receptor complex (IL12Rβ1 NM_005535.3 and IL12Rβ2 NM_001559.3). With this cell line, IL-12 activity can be monitored by measuring luciferase activity.

The functionality of the IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line was validated in dose-response assays using a recombinant human interleukin and inhibition assays using an IL-12 neutralizing antibody (#102108).

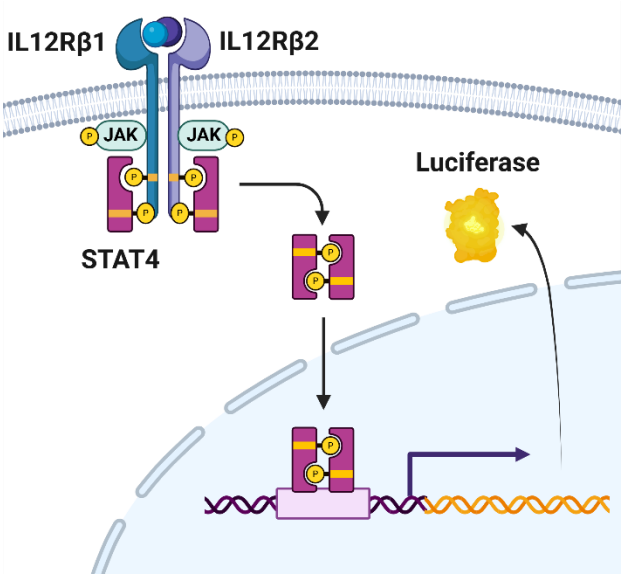

Figure 1. Illustration of the mechanism of action of the IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line.

Background

IL-12, a heterodimeric complex composed of p40 and p35, is a proinflammatory cytokine produced by activated macrophages and B-lymphocytes. This cytokine plays a crucial role in differentiating $CD4^+$ T-cell to T_H1 cells which promote cell-mediated immunity to intracellular pathogens by secreting several cytokines including IFN-γ (interferon gamma). In humans, the involvement of IL-12 in autoimmune diseases like Crohn's Disease (CD) and psoriasis has spurred significant efforts to develop reagents that antagonize its signaling pathway. However, recent research suggests that, paradoxically, stimulating IL-12 signaling could be a promising cancer immunotherapy strategy by stimulating both innate and adaptive immunity.

Application(s)

Screen and characterize anti-IL-12 antibodies as well as IL-12 mimetics

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)

Host Cell
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.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1Y	BPS Bioscience #82535
Cell Freezing Medium	BPS Bioscience #79796

Materials Required for Cellular Assay

Name	Ordering Information
IL-12, His-Tag recombinant	Thermo Fisher #200-12-H
Anti-IL-12 p40/ IL-12B Neutralizing Antibody	BPS Bioscience #102108
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 1Y (BPS Bioscience #82535):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 0.5 µg/ml of Puromycin, 100 µg/ml Hygromycin B, and 400 µg/ml G418.

Media Required for Functional Cellular Assay

Assay Medium: Thaw Medium 1

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 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 culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

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

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 1Y and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1Y.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 ~ 1:8 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 1Y 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 1~2 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.

Validation Data

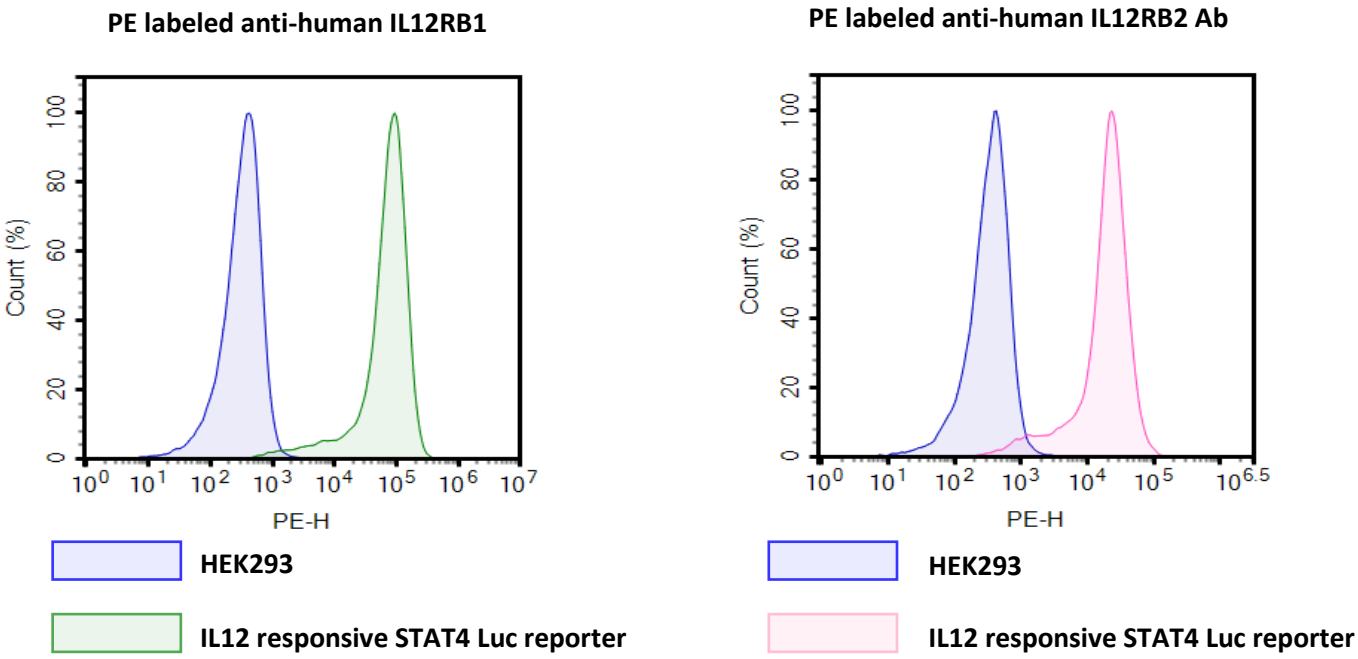

Figure 2. Expression analysis of IL12RB1 and IL12RB2 in the IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line by flow cytometry.
IL-12 Responsive STAT4 Luciferase Reporter HEK293 cells (green or pink) or control HEK293 cells (blue) were stained with Human IL-12 R beta 1 PE-conjugated Antibody (left; R&D System #FAB839P) and with PE anti-human IL12RB2 Antibody (right; Biolegend #394206) respectively, and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

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.
- Assay C should include “Cell-Free Control”, “Positive Control” (IL-12, no antibody), “Negative Control” (no IL-12, no antibody) and “Test Antibody” (IL-12, with antibody) conditions.

Assay Medium: Thaw Medium 1

A. 96-Well Assay Format: Dose-response of IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line to recombinant human IL-12

1. Seed IL-12 Responsive STAT4 Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 30,000 ~ 35,000 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-12 at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

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 ~10 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.

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

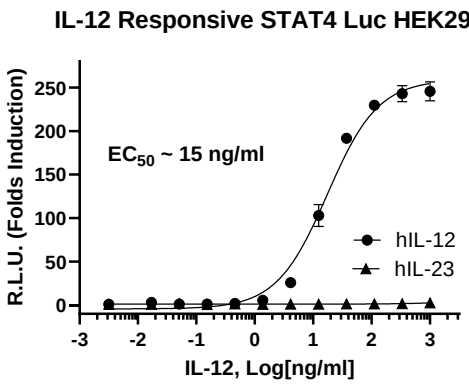

Figure 3. Dose response curve of IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line to recombinant human IL-12 in a 96-well assay format.
Cells were treated with increasing concentrations of IL-12 in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

B. 384-Well Assay Format: Dose-response of IL-12 Responsive STAT3 Luciferase Reporter HEK293 Cell Line to recombinant human IL-12

1. Seed IL-12 Responsive STAT3 Luciferase Reporter HEK293 cells into a white clear bottom, tissue culture treated 384-well microplate at a density of ~4,000 cells per well in 45 µl of Assay Medium. Leave 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-12 at concentrations 10-fold higher than the desired final concentrations in Assay Medium (5 µl/well).
4. Add 5 µl of each dilution to the "Stimulated" wells.
5. Add 5 µl of Assay Medium to the "Unstimulated Control" (negative control) wells.
6. Add 50 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 50 µl of the ONE-Step™ Luciferase reagent to each well.
9. Rock gently at RT for ~10 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.

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

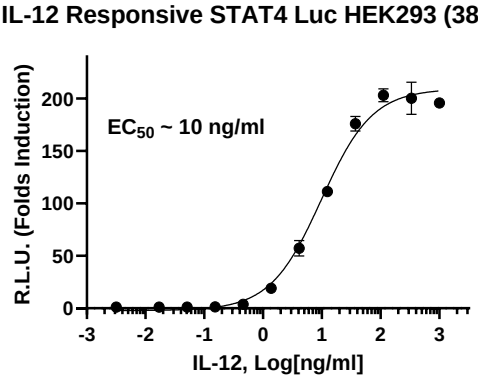

Figure 4. Dose response curve of IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line to recombinant human IL-12 in a 384-well assay format.
Cells were treated with increasing concentrations of IL-12 in a 384-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

C. Dose-response of IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line to an anti-IL12 antibody

1. Seed IL-12 Responsive STAT4 Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 30,000 ~ 35,000 cells per well in 80 µl of Assay Medium. Leave empty wells to determine the background luminescence ("Cell-Free Control").

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

3. The day of the experiment, preincubate IL-12 with anti-IL12 antibody:

   3.1 Prepare a serial dilution of anti-IL12 p40/ IL-12B neutralizing antibody (e.g. #102108) at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

   3.2 To each dilution of anti-IL12 antibody add an equal volume of Assay Medium containing the EC_{90} concentration of IL-12 (10 µl/well, to make 20 µl/well of Antibody + IL-12 Mixture).

   3.3 Pre-Incubate the Mixture at RT for 1 hour.

4. After the 1-hour pre- incubation of the mix:

   a. Add 20 µl of the Antibody+ IL-12 Mixture to the "Test Antibody" wells.

   b. Add 100 µl of Assay Medium to the "Cell-Free Control" wells.

   c. Add 20 µl of IL-12 solution only to the "Positive Control" wells.

   d. Add 20 µl of Assay Medium to the "Negative Control".

5. Incubate cells at 37°C in a CO_2 incubator for 5-6 hours.

6. After 5-6 hours, add 100 µl of the ONE-Step™ Luciferase reagent to each well.

7. Rock gently at RT for ~10 minutes.

8. Measure luminescence using a luminometer.

Data Analysis

Subtract the average luminescence of the "Negative Control" wells (no IL-12, no antibody) from the luminescence reading of all wells. The % luminescence is the average negative control-subtracted luminescence of the antibody treated wells divided by the average negative control-subtracted luminescence of the "Positive Control" wells (IL-12 only, no antibody) multiplied by 100.

$$\%\ Luminescence = \left(\frac{(luminescence\ of\ Test\ Antibody\ cells - average\ Negative\ Control)}{(average\ luminescence\ of\ Positive\ Control - average\ Negative\ Control)}\right) X100$$

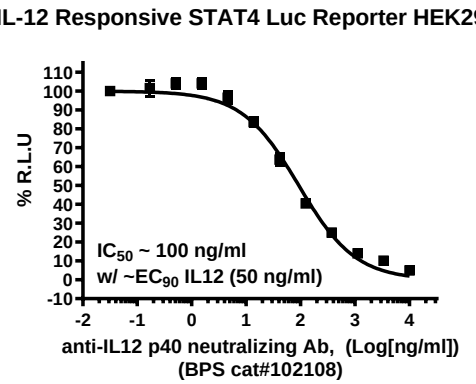

Figure 5. Inhibition of IL-12 induced reporter activity by Anti-IL-12 p40/ IL-12B Neutralizing Antibody in IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line.
Cells were treated with increasing concentrations of Anti-IL-12 p40/ IL-12B Neutralizing Antibody (#102108) as described in the protocol and incubated for 5-6 hours in a CO_2 incubator. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as % luminescence as described in the equation above.

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

Sequence

Human IL12RB1 sequence (NM_005535.3)

MEPLVTWVVPLLFLFLLSRQGAACRTSECCFQDPPYPDADSGSASGPRDLRCYRISSDRYECSWQYEGPTAGVSHFLRCCLSSGRC
CYFAAGSATRLQFSDQAGVSVLYTVTLWVESWARNQTEKSPEVTLQLYNSVKYEPPLGDIKVSKLAGQLRMEWETPDNQVGAE
VQFRHRTPSSPWKLGDCGPQDDDTESCLCPLEMNVAQEFQLRRRQLGSQGSSWSKWSSPVCVPPENPPQPQVRFSVEQLGQ
DGRRRLTLKEQPTQLELPEGCQGLAPGTEVTYRLQLHMLSCPCKAKATRTLHLGKMPYLSGAAYNVAVISSNQFGPGLNQTWHI
PADTHTEPVALNISVGTNGTTMYWPARAQSMTYCIEWQPVGQDGGLATCSLTAPQDPDPAGMATYSWSRESGAMGQEKCY
YITIFASAHPEKLTLWSTVLSTYHFGGNASAAGTPHHVSVKNHSLDSVSVDWAPSLLSTCPGVLKEYVVRCRDEDSKQVSEHPVQ
PTETQVTLSGLRAGVAYTVQVRADTAWLRGVWSQPQRFSIEVQVSDWLIFFASLGSFLSILLVGVLGYLGLNRAARHLCPPLPTPC
ASSAIEFPGGKETWQWINPVDFQEEASLQEALVVEMSWDKGERTEPLEKTELPEGAPELALDTELSLEDGDRCKAKM

Human IL12RB2 sequence (NM_001559.3)

MAHTFRGCSLAFMFIITWLLIKAKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFHYSRRNKLILYKFDRRINFHHGHSLNSQ
VTGLPLGTTLFVCKLACINSDEIQICGAEIFVGVAPEQPQNLSCIQKGEQGTVACTWERGRDTHLYTEYTLQLSGPKNLTWQKQCK
DIYCDYLDFGINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSN
SRLWNMVNVTKAKGRHDLLDLKPFTEYEFQISSKLHLYKGSWSDWSESLRAQTPEEEPTGMLDVWYMKRHIDYSRQQISLFWK
NLSVSEARGKILHYQVTLQELTGGKAMTQNITGHTSWTTVIPRTGNWAVAVSAANSKGSSLPTRINIMNLCEAGLLAPRQVSAN
SEGMDNILVTWQPPRKDPSAVQEYVVEWRELHPGGDTQVPLNWLRSRPYNVSALISENIKSYICYEIRVYALSGDQGGCSSILGN
SKHKAPLSGPHINAITEEKGSILISWNSIPVQEQMGCLLHYRIYWKERDSNSQPQLCEIPYRVSQNSHPINSLQPRVTYVLWMTAL
TAAGESSHGNEREFCLQGKANWMAFVAPSICIAIIMVGIFSTHYFQQKVFVLLAALRPQWCSREIPDPANSTCAKKYPIAEEKTQL
PLDRLLIDWPTPEDPEPLVISEVLHQVTPVFRHPPCSNWPQREKGIQGHQASEKDMMHSASSPPPPRALQAESRQLVDLYKVLES
RGSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDS
LML

References

Benson J. M., *et al.*, 2011 *Nat. Biotechnology.* 29(7): 615-624.

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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*
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials
IL-23 Responsive STAT3 Luciferase Reporter HEK293 Cell Line	82591	2 vials
IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line	78941	2 vials
IL15/IL15Ra Lentivirus	78938	500 µl x 2
Human Interleukin-15 Recombinant	90180	2 µg/10 µg
CRE/CREB Luciferase Reporter HEK293 Cell Line (cAMP/PKA Signaling Pathway)	60515	2 vials

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