SZABO SCANDIC

Part of Europa Biosite

Produktinformation

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

Weitere Information auf den folgenden Seiten!
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 STAT3 Reporter (eGFP)-HEK293 cell line is designed to monitor the STAT3 signal transduction pathway. It contains an eGFP gene driven by STAT3 response elements located upstream of the minimal TATA promoter. After activation by cytokines and growth factors, endogenous STAT3 binds to the DNA response elements, inducing transcription of the eGFP reporter gene.

Application

- Monitor the STAT3 signaling pathway activity
- Screen for activators or inhibitors of the STAT3 signaling pathway

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) 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 Assay

Name	Ordering Information
Human IL-6	R&D Systems #206-IL
Anti-IL-6R antibody	R&D Systems #MAB227
96-well tissue culture treated white clear-bottom assay plate	Corning #3610

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1N.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Invitrogen, #26140-079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01)

Growth Medium 1N (BPS Bioscience #79801):
Thaw Medium 1 (BPS Bioscience #60187) plus 0.5 µg/ml of Puromycin (InvivoGen, #ant-pr-1).

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

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Puromycin**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Puromycin**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.
4. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 1N (**contains Puromycin**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1N (contains Puromycin) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1N and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:5 to 1:10 weekly or twice a week.

Cell Freezing

1. To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Thaw Medium 1 (**no Puromycin**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

Inhibition of IL-6 induced STAT3 activity by anti-IL-6R antibody

1. Harvest STAT3 reporter (eGFP)-HEK293 cells and seed cells at a density of 20,000 cells per well into white clear bottom 96-well microplate in 80 µl of cell culture medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. The next day, add 10 µl of the desired concentration of anti-IL-6R antibody (R&D Systems, #MAB227) in cell culture medium to wells that will be stimulated by IL6 (final anti-IL-6R antibody= 2-20 nM). Incubate the cells at 37°C with 5% CO_2 for one hour. For control cells without antibody treatment, add 10 µl of fresh medium.

3.
   a. Add 10 µl of diluted human IL-6 in cell culture medium to wells labeled "IL6-stimulated" (final IL-6 concentration = 2 ng/ml).
   b. Add 10 µl of medium to the unstimulated control wells (for determining the unstimulated reporter activity).

4. Incubate the cells at 37°C with 5% CO_2 for 24-48 hours.

5. Observe the expression of eGFP under a fluorescence microscope (Ex/Em=488/510 nm).

IL-6 (2ng/ml)	-	+	+	+
Anti-IL-6R(nM)	0	0	2	20

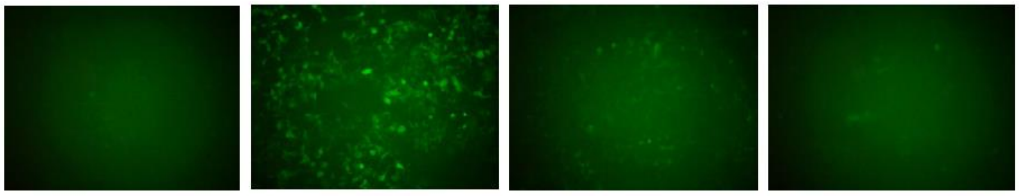

Figure 1. Inhibition of IL-6-induced Reporter Activity by Anti-IL-6R Antibody in STAT3 (eGFP) Reporter HEK293 Cells.

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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 Lentivirus	79744	2 x 500 µl
STAT3 eGFP Reporter Lentivirus	78197	2 x 500 µl
STAT3 Reporter Kit (STAT3 Signaling Pathway)	79730	500 rxns.
STAT3 Reporter (Luc)-HEK293 Cell line	79800-P	2 vials
Thaw Medium 1	60187-1	100 ml
Growth Medium 1N	79801	500 ml