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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

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

Recombinant HEK-293 cells expressing enhanced green fluorescent protein (eGFP) under the control of SRE responsive elements. This cell line is validated for its response to EGF or serum stimulation and to treatment with inhibitors of ERK signaling pathway.

Background

The MAPK/ERK signaling pathway is a key regulator of cell growth and differentiation. It can be activated by various extracellular stimuli including mitogens, growth factors, and cytokines. Upon stimulation, MEK1/2 phosphorylates and activates ERK1/2. The activated ERK translocates to the nucleus where it phosphorylates and activates transcription factors. The Ternary Complex Factors (TCFs), including Elk1, are among the best-characterized transcription factor substrates of ERK1/2. When phosphorylated by ERK1/2, Elk1 forms a complex with Serum Response Factor (SRF) and binds to Serum Response Element (SRE), resulting in the expression of numerous mitogen-inducible genes.

Application

- Screen for compound effect on the MAPK/ERK signaling pathway.
- Monitor MAPK/ERK signaling pathway activity and SRF-mediated activity.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Host Cell

HEK293

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 1J	BPS Bioscience #79552

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1J	BPS Bioscience #79552
Assay Medium 1B	BPS Bioscience #79617
Recombinant human EGF	BPS Bioscience #90201
U0126 (MEK inhibitor)	BPS Bioscience #27012

24-Well Tissue Culture Treated Plate
Round-Bottom 96 well plates or Round-Bottom Tubes
Flow Cytometry Cell Staining Buffer
Flow Cytometer or Fluorescence Microscope

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. BPS 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 (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Growth Medium 1J (BPS Bioscience #79552):
Thaw Medium 1 (BPS Bioscience #60187), 500 µg/ml of Geneticin/G418 (Thermo Fisher, #11811031)

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

Assay Medium 1B (BPS Bioscience #79617):
MEM medium (Thermo Fisher, #11095098) supplemented with 0.5% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to swirl the frozen cells for 30-40 seconds in a 37°C water-bath, then use 1-2 ml Thaw Medium 1 to completely thaw the cells. Transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin/G418**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin/G418**).
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 (**no Geneticin/G418**), 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 split before they are fully confluent. At first passage, switch to Growth Medium 1J (**contains Geneticin/G418**).

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 1J (**contains Geneticin/G418**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1J and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:6 to 1:8 weekly or twice per week.

Cell Freezing

1. To cryopreserve 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 Geneticin/G418**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796 or 10% DMSO + 90% FBS) 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

The following assays are designed for 24-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.

A. Response of SRE-eGFP Reporter HEK293 cells to EGF or Serum

1. Harvest SRE-eGFP Reporter HEK293 cells from culture in the Growth Medium 1J and seed cells at a density of ~50,000 cells per well into a 24-well tissue culture plate in 0.5 ml of Thaw Medium 1 (see above).
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. Remove medium from the wells and replace with 0.5 mL of Assay Medium 1B (contains 0.5% FBS).
4. Incubate cells at 37°C in a CO_2 incubator for 72 hours.
5. Remove medium from the wells and replace with desired doses of recombinant human EGF in Assay Medium 1B (0.5% FBS) or Assay Medium 1 (10% FBS).
6. Incubate cells at 37°C in a CO_2 incubator for 48 hours.
7. Analyze eGFP expression using a fluorescent microscope or flow cytometer (Ex/Em=488/510 nm)

D0	D1	D2	D3	D4	D5	D6
Plate Cells @ 50,000 cells/well	Treat with Assay Media 1B (0.5% FBS)			Treat with desired EGF and/or serum conditions		Analyze by microscopy or by flow cytometry

Experiment Timing: Response of SRE-EGFP Reporter HEK293 cells to EGF or Serum

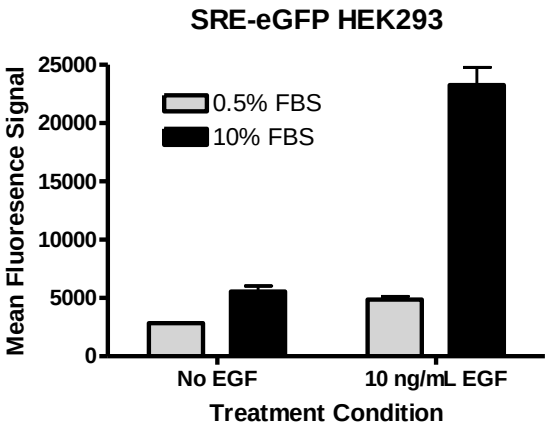

Figure 1. EGF or Serum induced the expression of SRE-eGFP reporter in SRE-eGFP Reporter HEK293. Data reported is from flow cytometry analysis and is presented as mean fluorescent intensity signal for the FITC channel across 3 replicates per condition.

B. Inhibition of EGF-induced reporter activity by inhibitor of ERK signaling pathway in SRE-eGFP Reporter – HEK293 cells

1. Harvest SRE-eGFP Reporter HEK293 cells from culture in the Growth Medium 1J and seed cells at a density of ~30,000 cells per well into a 24-well tissue culture plate in 0.5 ml of Thaw Medium 1 (see above). Plate enough wells for your dose curve of U0126 and appropriate controls with and without EGF.
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. Remove medium from the wells and replace with 0.5 ml of Assay Medium 1B (contains 0.5% FBS).
4. Incubate cells at 37°C in a CO_2 incubator for 72 hours.
5. Remove medium from the wells and replace Assay Medium 1B (contains 0.5% FBS) with dose curve of U0126 MEK inhibitor. Replace media in EGF/ No EGF control wells with Assay Medium 1B without inhibitor.
6. Incubate cells at 37°C in a CO_2 incubator for 24 hours.
7. Remove medium from wells and replace with dose curve of U0126 MEK inhibitor in Assay Medium 1 (contains 10% FBS). Replace media in EGF/ No EGF control wells with 10 ng/ml EGF and vehicle control respectively in Assay Medium 1 (contains 10% FBS).
8. Incubate cells at 37°C in a CO_2 incubator for 24 hours.
9. Analyze eGFP expression using a fluorescent microscope or flow cytometer (Ex/Em=488/510 nm)

D0	D1	D2	D3	D4	D5	D6
Plate Cells @ 30,000 cells/well	Treat with Assay Media 1B (0.5% FBS)			Pretreat with U0126 in Assay Media 1B (0.5% FBS)	Treat with EGF and U0126 Assay Media 1 (10% FBS)	Analyze by microscopy or by flow cytometry

Experiment Timing: Inhibition of EGF-induced reporter activity by inhibitor of ERK signaling pathway U0126 in SRE-eGFP Reporter – HEK293 cells

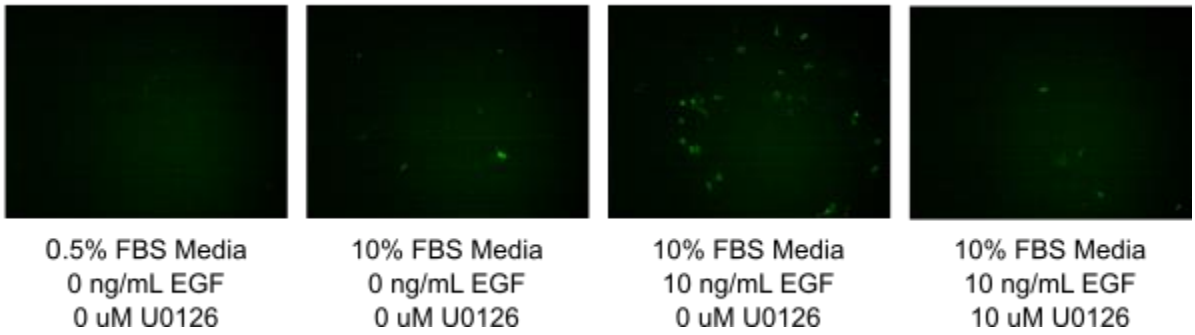

Figure 2. EGF or Serum induced the expression of SRE-eGFP reporter in SRE-eGFP Reporter HEK293. The expression of eGFP was inhibited by MEK inhibitor U0126.

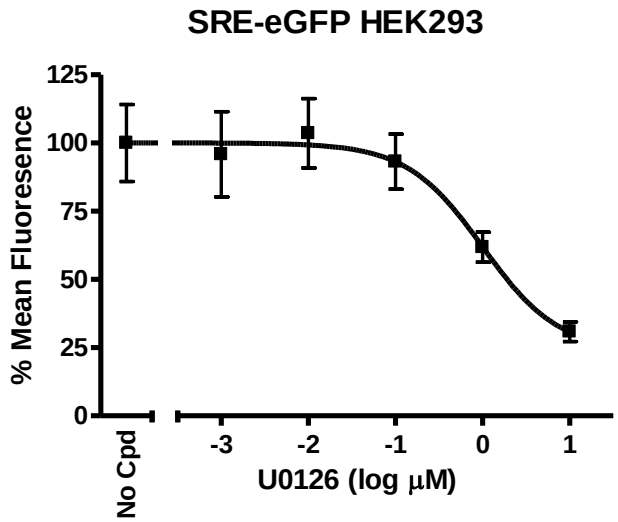

Figure 3. U0126 inhibition dose response curve. Data reported is from flow cytometry analysis and is the mean fluorescent intensity signal for the FITC channel across 3 replicates per condition. The results are shown as percentage of mean eGFP fluorescence normalized to cells treated with 10 ng/ml EGF in full serum media in the absence of U0126.

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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*
SRE Reporter – HEK293 Cell Line	60406	2 vials
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 reactions
Transfection Collection™ : SRE Transient Pack (MAPK/ERK Signaling Pathway)	79271	500 reactions
TrkA / SRE Reporter – HEK293 Recombinant Cell Line	79798	2 vials
CSF1R / SRE – Reporter HEK293 Recombinant Cell Line	79380	2 vials
CSF1R / SRE Reporter Kit (MAPK/ERK Signaling Pathway)	79379	500 reactions
ISRE Reporter-HEK293 Recombinant Cell Line (JAK pathway)	60510	2 vials
Thaw Medium 1	60187	100 ml
Growth Medium 1J	79552	500 ml