Description

The MAPK/ERK signaling pathway is a major participant in the regulation 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 TCFs (Ternary Complex Factors), including Elk1, are among the best-characterized transcription factor substrates of ERK. When phosphorylated by ERK, 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.

The SRE Reporter – HEK293 cell line contains a firefly luciferase gene under the control of SRE responsive elements stably integrated into HEK293 cells, resulting in an ERK pathway-responsive reporter cell line. This cell line is validated for the response to the stimulation of EGF or serum and to the treatment of inhibitors of ERK signaling pathway.

Application

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

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~1.5 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293

Mycoplasma Testing

The cell line has been screened using the PCR-based VenorGeM Mycoplasma Detection kit (Sigma-Aldrich) 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 1B	BPS Bioscience #79531

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531
Recombinant human EGF	BPS Bioscience #90201-1
U0126: Inhibitor of ERK pathway (MEK inhibitor). Prepare stock solution of U0126 in DMSO.	BPS Bioscience #27012
Assay Medium 1B	BPS Bioscience #79617
96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate	
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, 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 1B.

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 1B (BPS Bioscience #79531):
Thaw Medium 1 (BPS Bioscience, #60187) plus 400 µg/ml of Geneticin (Invitrogen #11811031)

Assay Medium 1B (BPS Bioscience #79617):
MEM medium (Hyclone #SH30024.01) supplemented with 0.5% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

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, transfer to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin**), spin down the cells, and resuspend the cells in pre-warmed Thaw Medium 1 (**no Geneticin**).
2. Transfer resuspended cells to a T75 flask and culture at 37°C in a CO_2 incubator.
3. At first passage, switch to Growth Medium 1B (**contains Geneticin**). Cells should be split before they reach complete confluence.

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, resuspend cells in Growth Medium 1B and transfer an aliquot of cell suspension into a new culture vessel.
2. Subcultivation ratio: 1:5 to 1:10 weekly.

Cell Freezing

1. To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. Add Growth Medium 1B and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS).
3. Freeze cells using a reduced rate freezing box (-0.5°C to -1°C per minute) at -80°C overnight, then move cells to liquid nitrogen for long term 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.

Functional Validation and Assay Performance

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

A. Response of SRE Reporter – HEK293 cells to EGF or serum

1. Harvest SRE Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells into a white clear-bottom 96-well microplate at a density of 30,000 cells per well in 100 µl of Thaw Medium 1.
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. The next day, carefully remove the medium from the wells. Add 50 µl of Assay Medium 1B to wells.
4. Incubate the plate at 37°C in a CO_2 incubator overnight.
5. The next day, add 50 µl of a serial dilution of human EGF or serum in Assay Medium 1B to stimulated wells.
   Add 50 µl of Assay Medium 1B to the unstimulated control wells.
   Add 100 µl of Assay Medium 1B to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.
6. Incubate the plate at 37°C in a CO_2 incubator for ~ 6 hours.

7. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.
8. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of SRE luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells

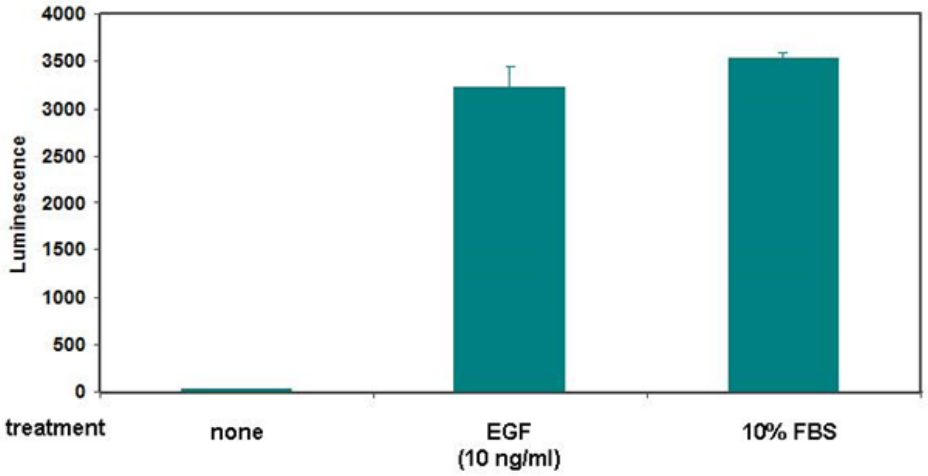

Figure 1. EGF or serum induced the expression of SRE reporter in SRE Reporter – HEK293.

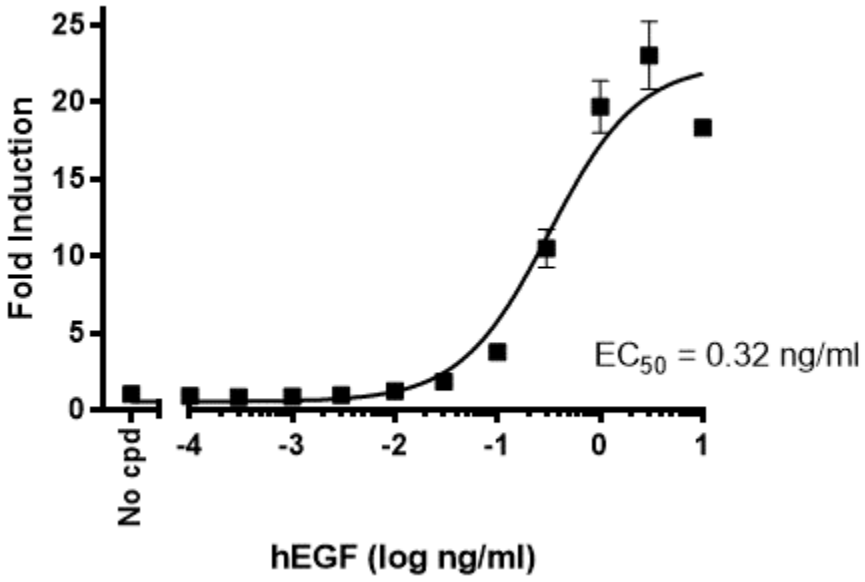

Figure 2. Dose response of SRE Reporter – HEK293 cells to EGF. The results are shown as fold induction of SRE luciferase reporter expression.

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

1. Harvest SRE Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells into a white clear-bottom 96-well microplate at a density of 30,000 cells per well in 100 µl of Thaw Medium 1.
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. The next day, carefully remove the medium from the wells. Add 40 µl of Assay Medium 1B to wells.
4. Dilute the inhibitor (U0126 or other test compounds) in Assay Medium 1B at 2x the final concentration and add 50 µl of compound dilution in Assay Medium 1B to the wells. The final concentration of DMSO in Assay Medium 1B can be up to 0.5%.
   Add 50 µl of Assay Medium 1B with same concentration of DMSO without inhibitor to control wells.
   Add 90 µl of Assay Medium 1B with DMSO to cell-free control wells (for determining background luminescence).
5. Incubate the plate at 37°C in a CO_2 incubator for overnight.
6. The next day, add 10 µls of diluted human EGF in Assay Medium 1B to stimulated wells (with and without inhibitor) (final [EGF] = 10 ng/ml).
   Add 10 µl of Assay Medium 1B to the unstimulated control wells (cells without inhibitor and EGF treatment for determining the basal activity).
   Add 10 µl of Assay Medium 1B to cell-free control wells.
   Set up each treatment in at least triplicate.

Treatment Reference Guide

	Stimulated Wells		**Unstimulated Control Wells**	**Cell-free Control Wells**
	With Inhibitor	**Without Inhibitor (control well)**		
Step 4	90 µl diluted inhibitor in Assay Medium 1B	90 µl Assay Medium 1B with DMSO only	90 µl Assay Medium 1B with DMSO only	90 µl Assay Medium 1B with DMSO only
Step 6	10 µl EGF in Assay Medium 1B (final [EGF] = 10 ng/ml)	10 µl EGF in Assay Medium 1B (final [EGF] = 10 ng/ml)	10 µl Assay Medium 1B	10 µl Assay Medium 1B

7. Incubate the plate at 37°C in a CO_2 incubator for ~6 hours.
8. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided:
   Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for 15-30 minutes.
   Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.
9. Data Analysis: Obtain background-subtracted luminescence by subtracting average background luminescence (cell-free control wells) from luminescence reading of all wells.

Figure 3. Inhibition of EGF-induced reporter activity by ERK pathway inhibitor in SRE Reporter – HEK293 cells

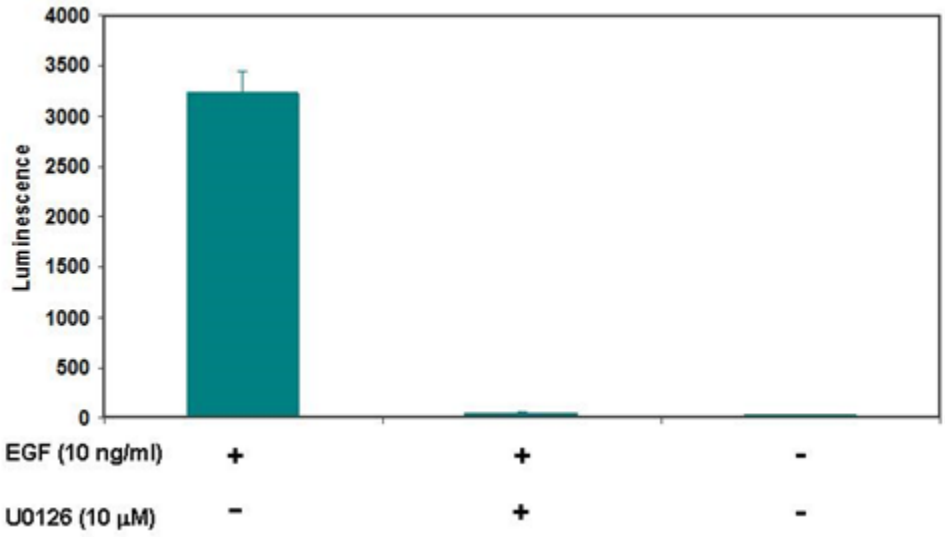

Figure 3a. U0126 blocked EGF-induced SRE reporter activity.

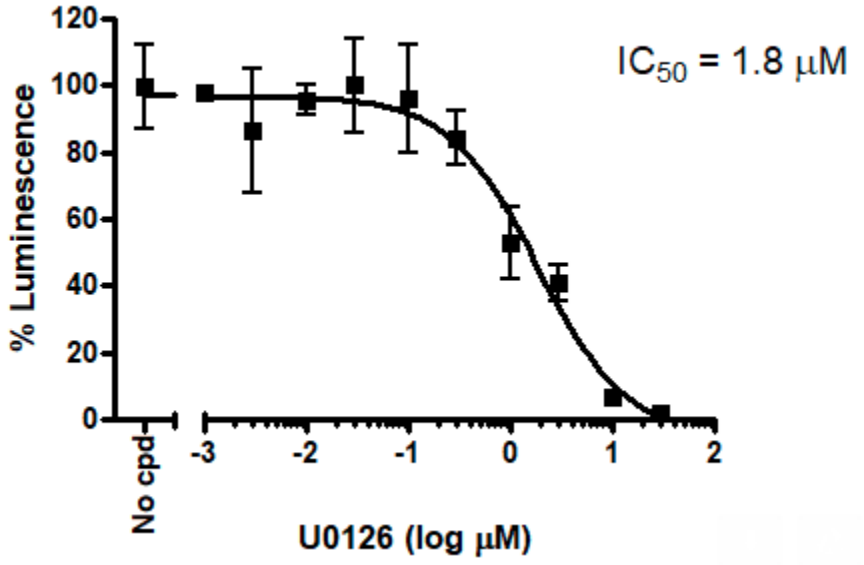

Figure 3b. U0126 inhibition dose response curve. The results were shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with EGF in the absence of U0126 was set at 100%.

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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:

1. Wong, K.K. (2009) Recent developments in anti-cancer agents targeting the Ras/Raf/ MEK/ERK pathway. Recent Pat. Anticancer Drug Discov. **4(1)**:28-35.
2. Treisman, R. (1992) The serum response element. Trends Biochem. Sci. **17(10)**: 423-426.

Related Products

Products	*Catalog #*	*Size*
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 reactions
FOXO Reporter Kit (PI3K/AKT Pathway)	60643	500 reactions
U0126	27012	5 mg
EGF, human	90201-1	100 µg
EGF, human	90201-2	500 µg
EGF, mouse	90200-1	100 µg
EGF, mouse	90200-2	500 µg
ERK1	40055	10 µg
ERK2	40299	10 µg
ONE-Step™ Luciferase Assay System	60690	Multiple Sizes