Data Sheet

MAPK/ERK Signaling Pathway
SRE Reporter – HEK293 Cell Line
Catalog #: 60406

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 activators or inhibitors of the MAPK/ERK signaling pathway.

Format
Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

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.

Storage
Immediately upon receipt, store in liquid nitrogen.

General culture conditions
Thaw Medium 1: MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

Growth Medium 1B: Thaw Medium 1 plus 400 µg/ml of Geneticin

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B.

It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

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 cells, and resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**. Transfer resuspended cells to a T25 flask and culture at 37°C in a CO_2 incubator. At first passage, switch to Growth Medium 1B **(contains Geneticin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 1B and transfer to a tube. Spin down cells, resuspend cells, and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:10 to 1:20 weekly.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1B and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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

Materials Required but Not Supplied

- Thaw Medium 1
- Growth Medium 1B
- Recombinant human EGF
- U0126: inhibitor of ERK pathway (MEK inhibitor). Prepare stock solution of U0126 in DMSO.
- Assay medium: MEM medium with 0.5% FBS, 1% non-essential amino acids, 1mM Na-pyruvate, and1% Pen/Strep
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System
- Luminometer

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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 the 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 wells. Add 45 µl of assay medium to wells.

4. Incubate the plate at 37°C in a CO_2 incubator for 18 to 24 hours.
5. The next day, add 5 µl of FBS or threefold serial dilution of human EGF in assay medium to stimulated wells.
   Add 5 µl of assay medium to the unstimulated control wells.
   Add 50 µl of assay medium 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.

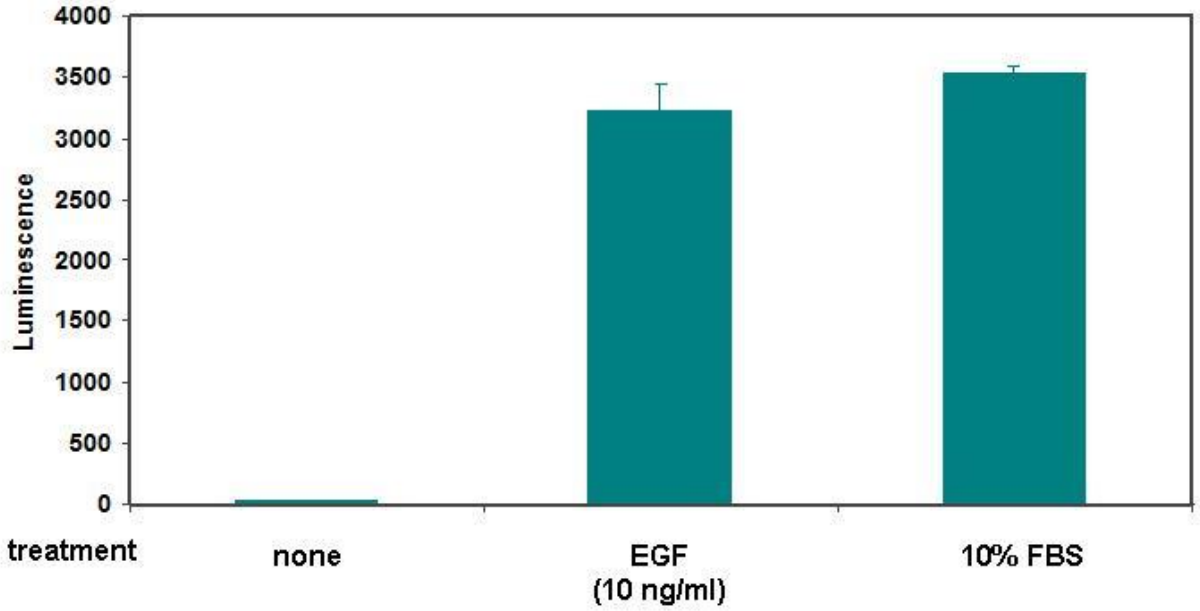
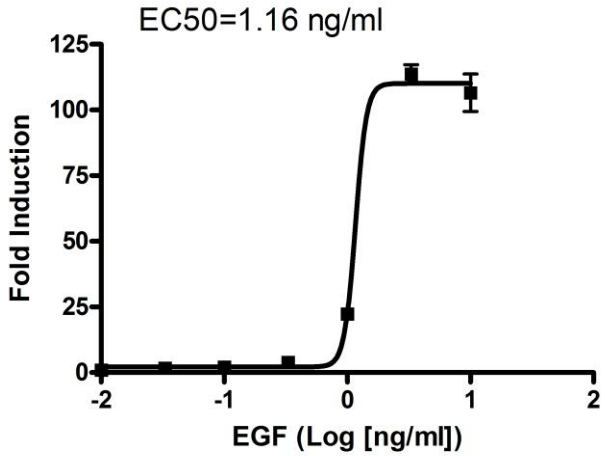


Figure 2. Dose response of SRE Reporter – HEK293 cells to EGF. The results were shown as fold induction of SRE luciferase reporter expression.



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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 the 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 for overnight.

3. The next day, dilute the inhibitor (U0126) stock in assay medium. Carefully remove the medium from wells and add 45 µl of diluted inhibitor in assay medium to the wells. The final concentration of DMSO in assay medium can be up to 0.5%.
   Add 45 µl of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
   Add 45 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).

4. Incubate the plate at 37°C in a CO_2 incubator for 18-24 hours.

5. The next day, add 5 µl of diluted human EGF in assay medium to stimulated wells (with and without inhibitor) (final [EGF] = 10 ng/ml).
   Add 5 µl of assay medium to the unstimulated control wells (cells without inhibitor and EGF treatment for determining the basal activity).
   Add 5 µl of assay medium 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 3	45 µl diluted inhibitor in assay medium	45 µl assay medium with DMSO only	45 µl assay medium with DMSO only	45 µl assay medium with DMSO only
Step 5	5 µl EGF in assay medium (final [EGF] = 10 ng/ml)	5 µl EGF in assay medium (final [EGF] = 10 ng/ml)	5 µl assay medium	5 µl assay medium

6. Incubate the plate at 37°C in a CO_2 incubator for ~6 hours.

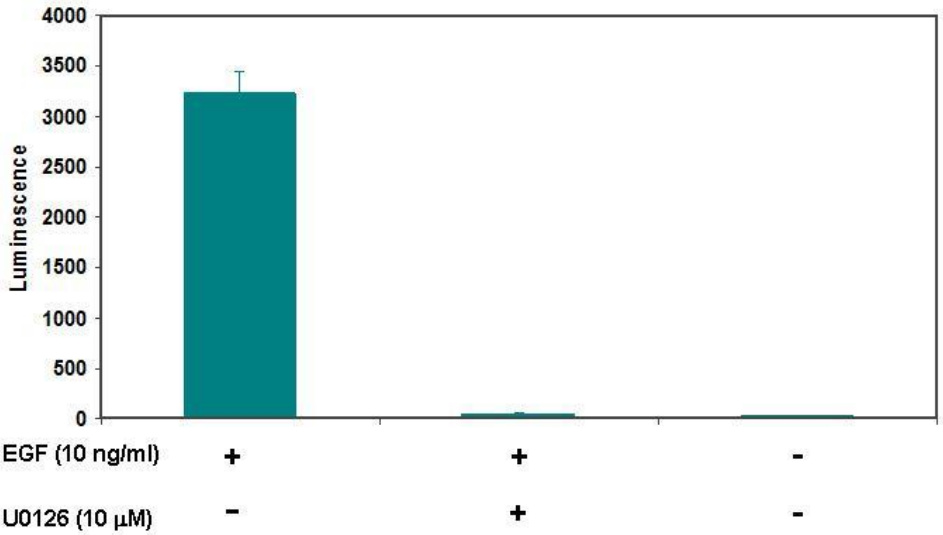
7. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.

If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.

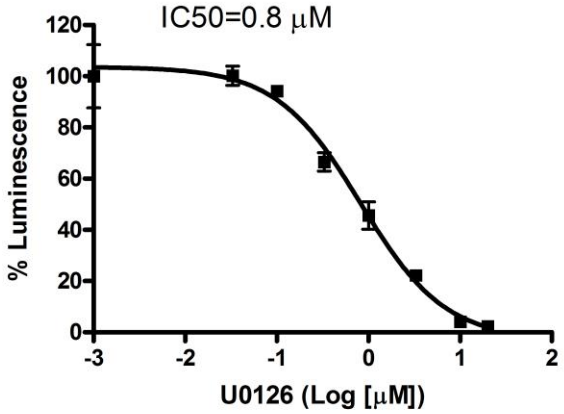
8. Data Analysis: Obtain background-subtracted luminescence by subtracting average background luminescence (cell-free control wells) from luminescence reading of all wells.

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

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



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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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.

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