6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

SRE Reporter Kit (MAPK/ERK Signaling Pathway)
Catalog #: 60511

Background

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 phosphorylate and activate 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.

Description

The *SRE Reporter Kit* is designed for monitoring the activity of the ERK signaling pathway and the transcriptional activity of SRF in cultured cells. The kit contains a transfection-ready SRE luciferase reporter vector, which is an ERK pathway-responsive reporter. This reporter contains the firefly luciferase gene under the control of multimerized SRE responsive elements located upstream of a minimal promoter. The SRE reporter is premixed with a constitutively-expressing *Renilla* luciferase vector that serves as an internal control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains the firefly luciferase gene under the control of a minimal promoter, without any additional response elements. The negative control is critical for determining pathway-specific effects and the background luciferase activity.

For assays in more difficult to transfect cell types, we suggest using our lentiviral products. The corresponding lentiviral product for this kit is BPS Bioscience #78627.

Applications

- Monitor MAPK/ERK signaling pathway activity and SRF-mediated activity.
- Screen for activators or inhibitors of the MAPK/ERK signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the MAPK/ERK pathway.

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	SRE luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 µl (55 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing *Renilla* luciferase vector	500 µl (55 ng DNA/ µl)	-20°C

Note: These vectors are designed for transient transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.]
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Dual Luciferase Assay System:
  Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000, follow the manufacturer's transfection protocol. Transfection conditions should be optimized according to the cell type and study requirements.

All amounts and volumes in the following setup are given on a per-well basis.

1. One day before transfection, seed cells at a density of ~ 30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

   a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently.

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Depending upon the experimental design, the DNA mixtures may be any of following combinations:

- **1 µl of Reporter** (component A); in this experiment, the control transfection is **1 µl of Negative Control Reporter** (component B).
- **1 µl of Reporter** (component A) + experimental vector expressing gene of interest; in this experiment, the control transfections are: **1 µl of Reporte**r (component A) + negative control expression vector, **1 µl of Negative Control Reporter** (component B) + experimental vector expressing gene of interest, and 1 **µl of Negative Control Reporter** (component B) + negative control expression vector.
- **1 µl of Reporter** (component A) + specific siRNA; in this experiment, the control transfections are: **1 µl of Reporte**r (component A) + negative control siRNA, **1 µl of Negative Control Reporter** (component B) + specific siRNA, and **1 µl of Negative Control Reporter** (component B) + negative control siRNA.

Note: we recommend setting up each condition in at least triplicate, and preparing transfection cocktail for multiple wells to minimize pipetting errors.

b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.

Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of the complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a CO_2 incubator. After ~5 to 6 hours of transfection, change medium to fresh medium with 0.5% serum. Incubate cells at 37°C in a CO_2 incubator overnight.

5. The next day, induce the SRE reporter with medium containing activators of the ERK pathway such as high percentage of serum or growth factors. Incubate cells at 37°C in a CO_2 incubator for ~ 6 hours. After 6-hour treatment, perform the Dual Luciferase Assay System following the protocol on the BPS data sheet (BPS Cat. #60683).

To study the effect of inhibitors on the ERK pathway, after ~5-6 hours of transfection, treat cells with inhibitors in medium containing 0.5% serum. The next day, treat cells with activators for 6 hours, then perform the luciferase assay.

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Sample protocol to determine the effect of serum or EGF on SRE reporter activity in HEK293 cells

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of growth medium (MEM/EBSS (Hyclone #SH30024.01), 10% FBS, 1% non-essential amino acids, 1 mM Na-pyruvate, 1% Pen/Strep). Incubate cells overnight at 37°C in a CO_2 incubator.

2. The next day, transfect 1 µl of SRE reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~ 6 hours of transfection, change medium to 50 µl of medium containing 0.5% FBS (MEM, 0.5% FBS, with non-essential amino acids, Na-pyruvate, and 1% Pen/Strep). Incubate cells at 37°C in a CO_2 incubator for ~ 16 to 18 hours.

4. The next day after transfection, treat cells with 50 µl of medium containing a high percentage of FBS, with or without EGF, or medium containing 0.5% FBS with EGF. For unstimulated control wells, use cells in medium with 0.5% FBS. Add 50 µl of growth medium to cell-free control wells to determine the background luminescence). Set up each treatment in at least triplicate.

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

6. After ~48 hours of transfection, perform dual luciferase assay using BPS Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

7. To obtain the normalized luciferase activity for the SRE reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from SRE reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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Figure 1. Serum induced the expression of SRE reporter. The results are shown as fold induction of normalized SRE reporter activity. Fold induction is determined by comparing values against the mean value for control cells with 0.5% FBS treatment.

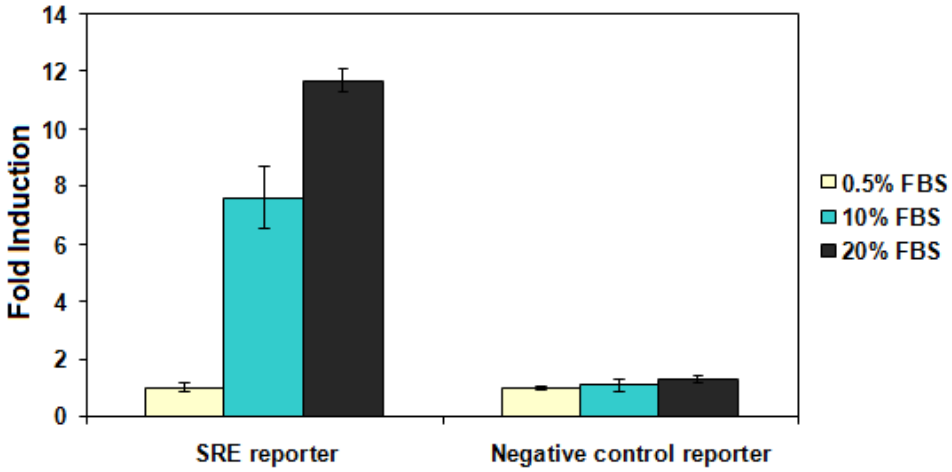

Figure 2. EGF induced the expression of SRE reporter. The results are shown as fold induction of normalized SRE reporter activity. Fold induction are determined by comparing values against the mean value for control cells with 0.5% FBS treatment only.

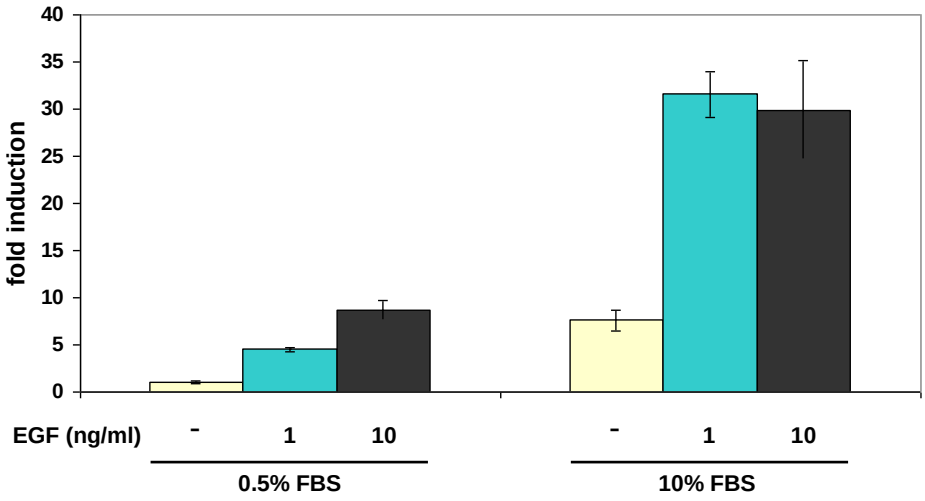

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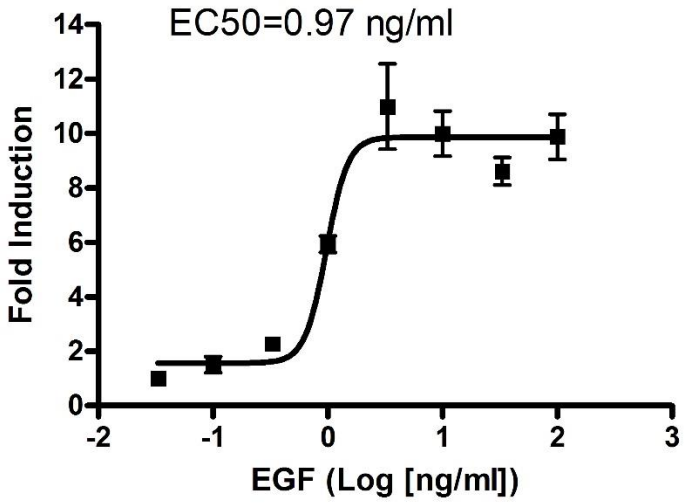
Figure 3. Dose response of SRE reporter activity to EGF in the presence of 0.5% FBS. The results are shown as fold induction of normalized SRE reporter activity. Fold induction is determined by comparing values against the mean value for control cells without EGF treatment.

The EC50 of EGF is ~0.97 ng/ml



Sample protocol to determine the effect of inhibitors of the ERK pathway on SRE reporter activity in HEK293 cells

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of growth medium. Incubate cells overnight at 37°C in a CO_2 incubator.

2. The next day, transfect 1 µl of SRE reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~6 hours of transfection, treat transfected cells with three-fold serial dilution of U0126 (MEK inhibitor) in 50 µl of medium containing 0.5% FBS. For wells without U0126, treat cells with medium containing 0.5% FBS only. Incubate cells at 37°C in a CO_2 incubator for ~ 16 to 18 hours.

4. The next day after transfection, treat the cells with recombinant EGF (final concentration 10 ng/ml) in 50 µl of medium containing 0.5% FBS with U0126. For unstimulated control wells, determine the basal activity using cells in medium with 0.5% FBS. To determine background luminescence, ; add 50µl of medium to cell-free control wells. Set up each treatment in at least triplicate.

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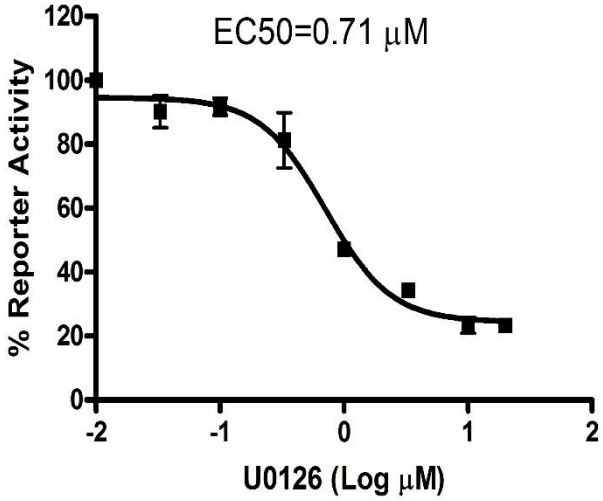
5. Incubate cells at 37°C in a CO_2 incubator for ~ 6 hours.

6. After ~48 hours of transfection, perform dual luciferase assay using BPS Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

7. To obtain the normalized luciferase activity for the SRE reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from the SRE reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 4. Inhibition of EGF-induced SRE reporter activity by ERK pathway inhibitor, U0126. The results are shown as percentage of SRE reporter activity. The normalized luciferase activity for cells stimulated with EGF in the absence of U0126 is set at 100%.

The IC50 of U0126 is ~ 0.7 µM



References

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.
Treisman, R. (1992) The serum response element. *Trends Biochem Sci*. **17(10):** 423-426.

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Related Products

Product Name	Catalog #	Size
Dual Luciferase (Firefly-Renilla) Assay System	60683-1	10 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-2	100 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-3	1 L
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
MAP3K14 (NIK)	40090	10 µg
MAPKAPK2 (MK2)	40088	100 µg
MAPK10 (JNK3)	40092	10 µg
MEK1 (K97R)	40075	100 µg
MEK1, mouse	40121	10 µg
MEK1, human	40123	10 µg
MEK1, GST-tag	40527	50 µg
MEK2	40125	10 µg
MEKK2	40122	10 µg
MEKK3	40124	10 µg
U0126	27012	5 mg