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Lieferung & Zahlungsart

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- Trockeneiszuschlag
- Gefahrgutzuschlag
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Data Sheet

The Transfection Collection™ – SRE Transient Pack
(MAPK/ERK Signaling Pathway)
Catalog #: 79271

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 Transient Pack is designed to provide the tools necessary for transiently transfecting and monitoring the activity of the ERK signaling pathway and the transcriptional activity of SRE in cultured HEK293 cells. The kit contains transfection-ready vectors containing firefly luciferase as a MAPK/ERK pathway-responsive reporter and constitutively expressing Renilla luciferase as a transfection control. It also includes the Dual Luciferase detection reagents to detect both luciferase activities and specialized medium for growing and assaying HEK293 cells.

The key to the SRE Transient Pack is the 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 pack 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.

Additionally, the pack includes cell culture medium (BPS Medium 1) that has been optimized for use with HEK293 cells*. BPS Medium 1 includes MEM medium, 10% fetal bovine serum, 1% non-essential amino acids, sodium pyruvate, and 1% Pen/Strep. Finally, the pack provides the Dual Luciferase (Firefly-Renilla) Assay System. These luciferase reagents provide highly sensitive, stable detection of firefly luciferase activity and Renilla luciferase activity. The dual

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luciferase reagents can be used directly in cells in growth medium, and can be detected with any luminometer; automated injectors are not required.

* Other mammalian cell lines may also be used, but an alternate cell culture medium may be required for optimal cell growth.

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.

Components

Component	Amount	Storage
Reporter (Component A) SRE luciferase reporter vector* + constitutively expressing Renilla luciferase vector*	500 µl (60 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B) Non-inducible luciferase vector*+ constitutively expressing Renilla luciferase vector*	500 µl (60 ng DNA/ µl)	-20°C
Firefly Luciferase Reagent Buffer	10 ml	-20°C
Firefly Luciferase Reagent Substrate (100x)	100 µl	-20°C *Protect from light*
Renilla Luciferase Reagent Buffer	10 ml	Room Temp.
Renilla Luciferase Reagent substrate (100x)	100 µl	-20°C *Protect from light*
BPS Medium 1	100 ml	+4°C

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

Materials Required but Not Supplied

- HEK293 cells. Other mammalian cell lines may also be used, but an alternate cell culture medium may be required for optimal cell growth.
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate (Corning, #3610)
- 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) (assay medium)
- Luminometer

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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 cultre 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 provided 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 BPS Medium 1 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. 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. Carefully remove and discard 30 µl of media from each of the wells of cell culture, taking care not to disturb the cells or touch the bottom of the well with the pipet tip. Add the 30 µl

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of the complexes to each well containing 70 µl 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 Opti-MEM I Reduced Serum 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 up to 20% (e.g. BPS Medium 1 contains 10% FBS) 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 (below).

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.

Dual Luciferase Assay Procedure

1. Thaw Firefly Luciferase Reagent Buffer by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. Note: It is important that the Firefly Luciferase Reagent Buffer be at room temperature before use.

2. Calculate the amount of Firefly Luciferase Assay Working solution needed for the experiment (Firefly Luciferase Reagent Buffer + Firefly Luciferase Reagent Substrate). Immediately prior to performing the experiment, prepare the Firefly Luciferase Assay Working Solution by diluting Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer at a 1:100 ratio and mix well. Avoid exposing to excessive light. Only use enough of each component for the experiment, remaining Firefly Luciferase Reagent Buffer and Firefly Luciferase Reagent Substrate should be stored separately at -20°C.

3. Remove multi-well plate containing mammalian cells from incubator. Note: plates must be compatible with luminescence measurement by luminometer being used.

4. Add equal volume of Firefly Luciferase Assay Working Solution (step 2) to the culture medium in each well. Example: 96-well plate with 100 µl of culture medium requires 100 µl of Firefly Luciferase Assay Working Solution per well.

Gently rock the plates for ~15 minutes at room temperature. Measure firefly luminescence using a luminometer. The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

5. Calculate the amount of Renilla Luciferase Assay Working Solution needed for the experiment (Renilla Luciferase Reagent Buffer + Renilla Luciferase Reagent Substrate). Prepare the Renilla Luciferase Assay Working Solution by diluting Renilla Luciferase Reagent Substrate into Renilla Luciferase Reagent Buffer at a 1:100 ratio and mix well. Avoid exposing to excessive heat or light. Only use enough of each component for the experiment.

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6. Add equal volume of Renilla Luciferase Assay Working Solution (step 5) to each well. Example: 96-well plate with 100 µl of culture medium + 100 µl Firefly Luciferase Reagent requires 100 µl of Renilla Luciferase Assay Working Solution per well.

7. Gently rock the plates for ~1 minute at room temperature. Measure Renilla luminescence using a luminometer.

8. Data analysis: subtract background (wells with medium and luciferase reagent only) from all the readings. 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. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

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 Thaw Medium 1. 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 Opti-MEM I Reduced Serum Medium containing 0.5% FBS. 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 up to 20% (e.g. BPS Medium 1 contains 10% FBS), with or without EGF, or medium containing 0.5% FBS with EGF. For unstimulated control wells, use cells in same medium with 0.5% FBS. Add 50 µl of same 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 as described above in **Dual Luciferase Assay Procedure**. Briefly, dilute 100x **Firefly Luciferase Reagent Substrate** into **Firefly Luciferase Reagent Buffer** that has been equilibrated to room temperature. 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** into **Renilla Luciferase Reagent Buffer**. Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

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7. Data analysis: subtract background (wells with medium and luciferase reagent only) from all the readings. 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. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

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.

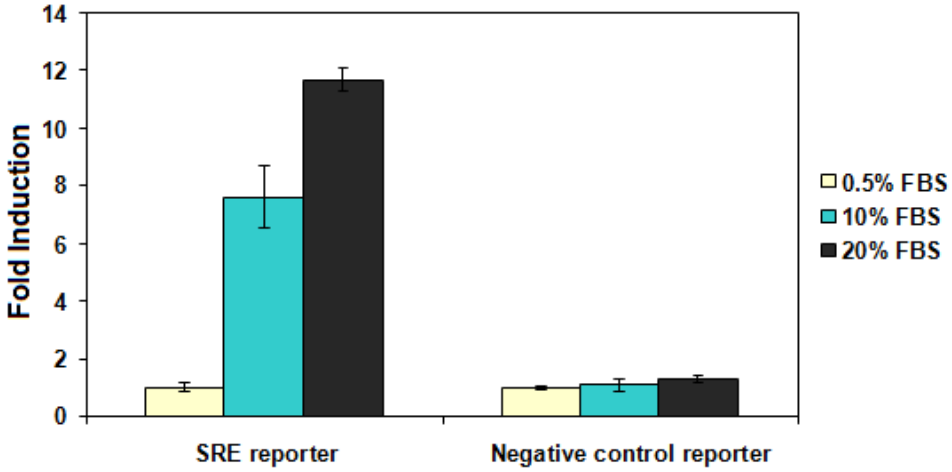

Figure 2. EGF induced the expression of SRE reporter. The results are shown as fold induction of normalized SRE reporter activity. Fold induction are determined by comparing values against the mean value for control cells with 0.5% FBS treatment only.

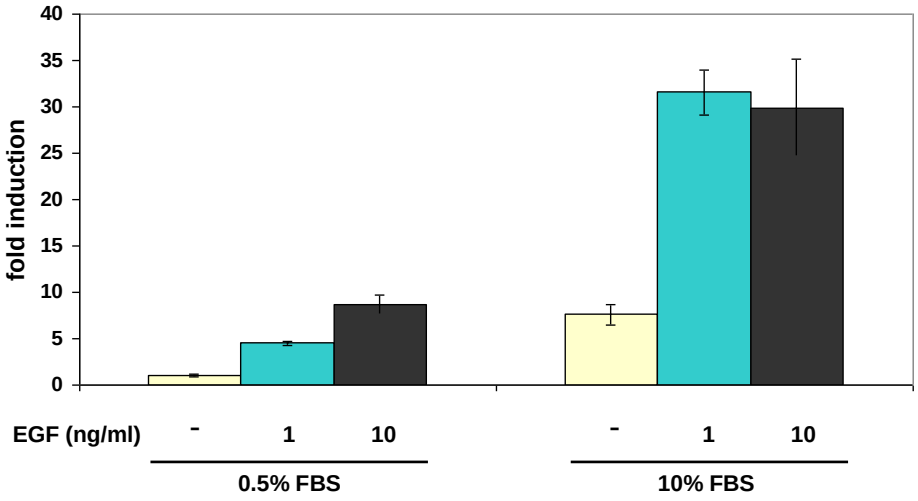

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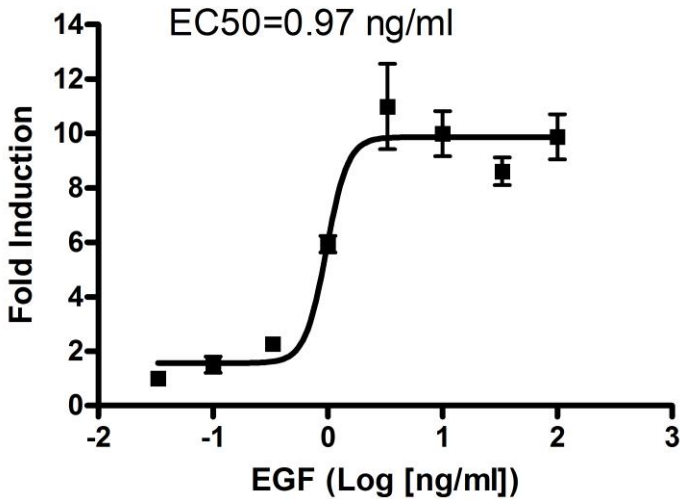
Figure 3. Dose response of SRE reporter activity to EGF in the presence of 0.5% FBS. The results are shown as fold induction of normalized SRE reporter activity. Fold induction is determined by comparing values against the mean value for control cells without EGF treatment.

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 BPS Medium 1. 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 Opti-MEM 1 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

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background luminescence, add 50 µl of medium to cell-free control wells. Set up each treatment in at least triplicate.

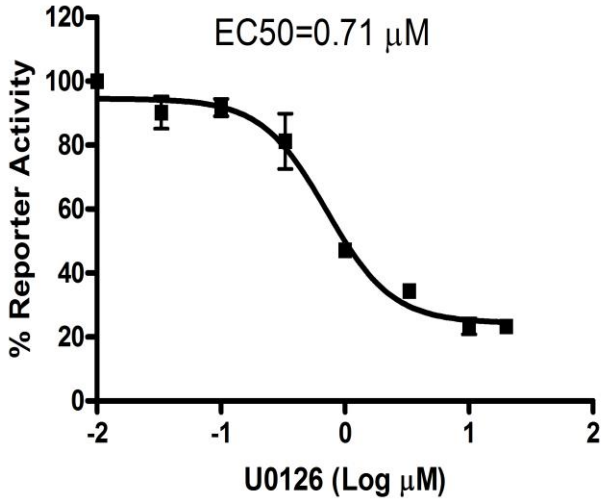
5. Incubate cells at 37°C in a CO_2 incubator for ~ 6 hours.

6. After ~48 hours of transfection, perform dual luciferase assay as described above in **Dual Luciferase Assay Procedure**. Briefly, dilute 100x Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer. 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 into Renilla Luciferase Reagent Buffer. 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



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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.

Refills

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
BPS Medium 1	79259	100 ml
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 rxns

Related Products

Product Name	Catalog #	Size
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