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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

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

CSF1R / SRE Reporter Kit (MAPK/ERK Signaling Pathway)
Catalog #: 79379

Background

Colony Stimulating Factor 1 Receptor (CSF1R, CD115, M-CSF-R) is the receptor for the cytokine CSF1 (MCSF; CSF-1), which controls the growth, function, and differentiation of macrophages. This interaction drives the development and survival of Tumor-Associated Macrophages (TAMs), which in turn suppress the local immune response to the cancer. An alternative ligand is IL-34, which has been implicated in neuroprotective and survival signals following brain injury and neurodegeneration.

As a tyrosine kinase transmembrane receptor, activation of CSF1R by its ligand initiates a vast array of intracellular activity, including activation of the MAPK/ERK signaling pathway. 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 *CSF1R SRE Reporter Kit* is designed for monitoring the activity of the CSF1R signaling pathway in cultured cells. The kit contains a transfection-ready vector for CSF1R and SRE luciferase reporter vector. Upon ligand binding, active CSF1R will initiate the MAPK/ERK signaling pathway leading to expression of the SRE-controlled 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.

Applications

- Monitor CSF1R/MAPK/ERK signaling pathway activity and SRF-mediated activity.
- Screen for activators or inhibitors of the CSF1R/MAPK/ERK signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the CSF1R/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 (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
CSF1R Expression Vector (Component C)	CSF1R expression vector	2 x 250 µL (100 ng DNA/µL)	-20°C
Negative Control Expression Vector (Component D)	Empty expression vector without CSF1R	2 x 250 µL (100 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) + Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062). However, other transfection reagents work equally well.]
- Recombinant Human CSF1 (BPS cat #90215-B) or Recombinant Human IL34 (Biolegend cat# 577904)
- BPS Medium 8 (BPS Cat. #79385), or another low-serum assay medium
- 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,

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Fax: 1.858.481.8694
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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. 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) + **1 µL CSF1R Expression Vector** (component C); in this experiment, the control transfections are: **1 µL of Reporter** (component A) + **1 µL Negative Control Expression Vector** (component D), **1 µL of Negative Control Reporter** (component B) + **1 µL CSF1R expression vector** (component C), and **1 µL of Negative Control Reporter** (component B) + **1 µL Negative Control Expression vector** (component D).
   - **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.

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4. Incubate cells at 37°C in a CO_2 incubator. After ~5 to 6 hours of transfection, change medium to BPS Medium 8 (BPS Cat. #79385), or growth medium with 0.5% serum. Incubate cells at 37°C in a CO_2 incubator overnight.

5. The next day, induce the SRE reporter by activating CSF1R with either recombinant CSF1 or IL-34. 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 CSF1R, pre-treat cells with inhibitors in BPS Medium 8 or medium containing 0.5% serum for greater than 30 minutes. Next treat cells with CSF1R ligand for 6 hours, then perform the luciferase assay.

Sample protocol to determine the effect of recombinant CSF1 or IL-34 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) and 1 µL CSF1R expression vector (component C) into each well following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~ 6 hours of transfection, change medium to 90 µL of BPS Medium 8 (BPS Cat. #79385) or growth 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 various doses of recombinant human CSF1 or IL-34 in 10 µL of BPS Medium 8 or medium containing 0.5% FBS. For unstimulated control wells, add only medium. Use 100 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 100 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).

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Add 100 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.

Figure 1. CSF1 and IL34 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.

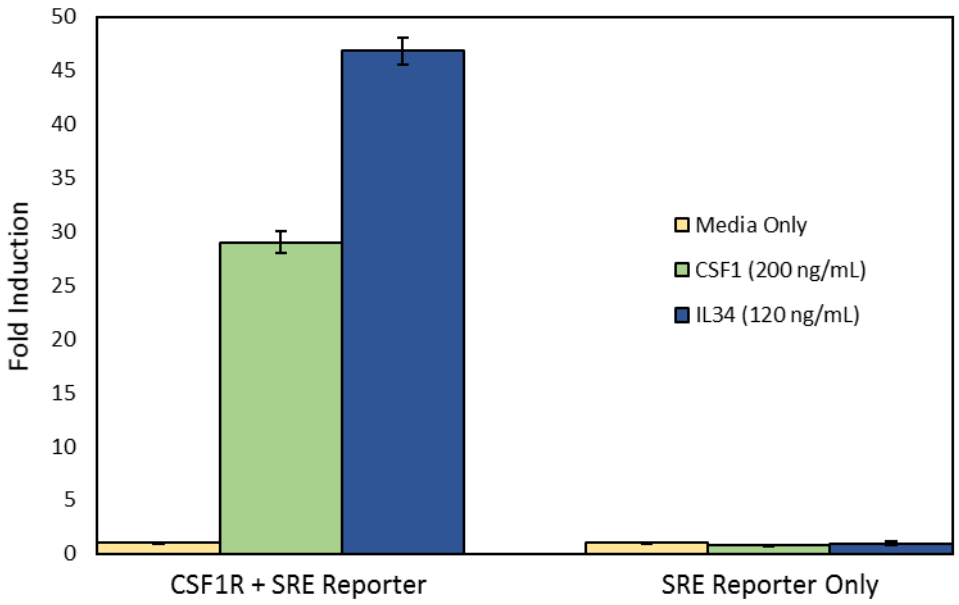

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Figure 2. Dose response of SRE reporter activity to CSF1 in the presence of 0.5% FBS. The results are shown as fold induction of normalized SRE reporter activity. Fold induction is determined by comparing values against the mean value for control cells with 0.5% FBS treatment. The EC50 of CSF1 is ~6.28ng/mL.

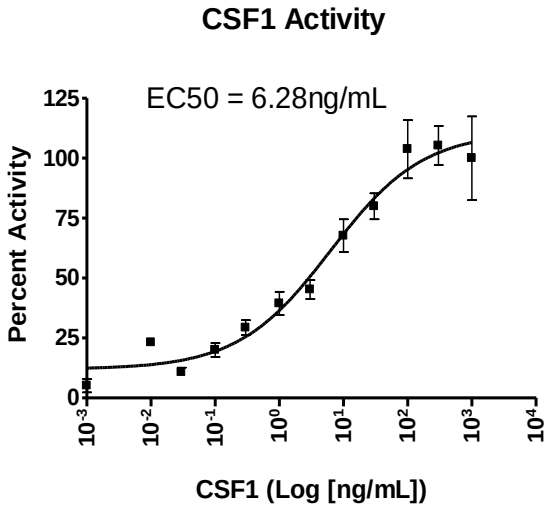
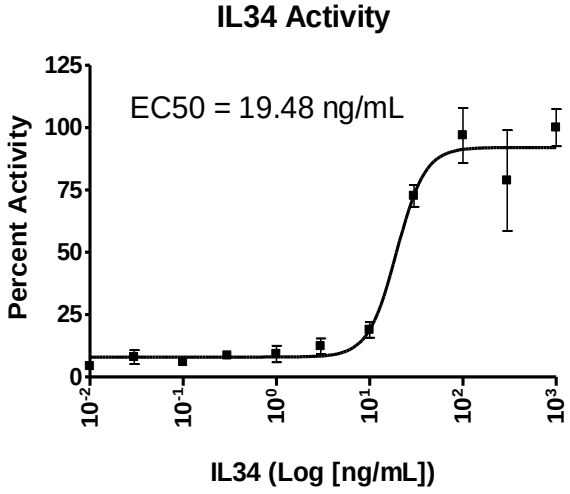


Figure 3. Dose response of SRE reporter activity to IL-34 in the presence of 0.5% FBS. The results are shown as fold induction of normalized SRE reporter activity. Fold induction is determined by comparing values against the mean value for control cells with 0.5% FBS treatment. The EC50 of IL-34 is ~19.48ng/mL.



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Sample protocol to determine the effect of inhibitors of the CSF1R 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) and 1 µL CSF1R expression vector (component C) into each well following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~ 6 hours of transfection, change medium to 80 µL of BPS Medium 8 (BPS Cat. #79385) or growth medium containing 0.5% FBS. Incubate cells at 37°C in a CO_2 incubator for ~ 16 to 18 hours.

4. The next day, treat transfected cells with three-fold serial dilution of Pexidartinib (PLX3397) in 10 µl of medium containing 0.5% FBS. For wells without Pexidartinib, treat cells with medium containing 0.5% FBS only. Incubate cells at 37°C in a CO_2 incubator for ~30 minutes.

5. After incubation, treat the cells with recombinant CSF1 or IL34 (final concentration 10 ng/ml and 20 ng/mL, respectively) in 10 µl of medium containing 0.5% FBS. For unstimulated control wells, determine the basal activity using cells in medium with 0.5% FBS. To determine background luminescence, add 100µl of medium to cell-free control wells. Set up each treatment in at least triplicate.

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

7. 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 100 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 100 µL of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

8. 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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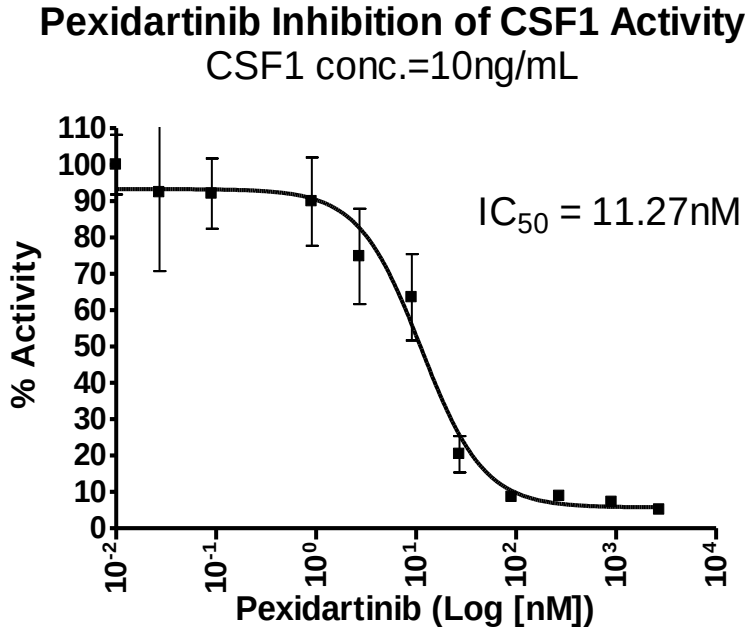
Figure 4. Inhibition of CSF1-induced SRE reporter activity by CSF1R pathway inhibitor, PLX3397. The results are shown as percentage of SRE reporter activity. The normalized luciferase activity for cells stimulated with CSF1 in the absence of PLX3397 is set at 100%.

The IC50 of PLX3397 is 11.27nM.



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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
Human Macrophage Colony Stimulating Factor	90215-A	2µg
Human Macrophage Colony Stimulating Factor	90215-B	10 µg
SRE Reporter Kit	60511	500 reactions
CSF1R, Fc-Fusion, Avi-Tag, Biotin-Labeled	79343	50 µg
CSF1R, Fc-Fusion, Avi-Tag (Human) HiP™	72550	100 µg
CSF1R, untagged (FMS, CD115)	79110	10 µg
CSF1R, GST-tag	40227	10 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