Description

The CSF1R / SRE – Reporter HEK293 Recombinant Cell Line has been transfected with full-length human CSF1R cDNA (NP_005202) under a CMV promoter for high constitutive expression. The SRE–luciferase reporter is also stably integrated into the genome. The firefly luciferase gene is controlled by 4 copies of the Serum Response Element upstream of a minimal promoter. Upon ligand binding, active CSF1R will initiate the MAPK/ERK signaling pathway, leading to expression of the SRE-controlled luciferase reporter.

Background

Colony Stimulating Factor 1 Receptor (CSF1R, CSFR, CD115, M-CSF-R) is a single-pass tyrosine kinase transmembrane receptor which is part of the type III protein tyrosine kinase receptor family. CSF1R is activated by either of two cytokines, CSF1 (MCSF; CSF-1) and IL-34 (IL34), causing homodimerization and activation of downstream kinase activity. CSF1R is expressed on the surface of monocytes and macrophages, and its activation controls the growth, function, and differentiation of macrophages. This interaction is used by numerous cancer types, such as diffuse-type tenosynovial giant cell tumors (dt-GCT), to evade the immune system. By overexpressing the cytokine CSF1, these cells drive the development and survival of Tumor-Associated Macrophages (TAMs), which in turn suppress the local immune response to the cancer.

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.

Application

- Monitor the CSF1R/MAPK/ERK signaling pathway activity and SRF-mediated activity.
- Screen for compound activity of the CSF1R/MAPK/ERK signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 X 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Host Cell

HEK293

Mycoplasma Testing

The cell line has been screened 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 1M	BPS Bioscience #79723

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1M	BPS Bioscience #79723
Assay Medium 1B	BPS Bioscience #79617
Recombinant human M-CSF or Recombinant human IL-34	BioLegend #574802 or R&D Systems #5265-IL/CF
Pexidartinib/PLX3397	SelleckChem #S7818
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. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Growth Medium 1M (BPS Bioscience #79723):
Thaw Medium 1 (BPS Bioscience #60187), 400 µg/ml of Geneticin (Thermo Fisher #11811031) and 0.5 µg/ml Puromycin Dihydrochloride (Thermo Fisher #A1113803).

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 or Puromycin**), spin down cells at 1000 rpm, and resuspend cells in 10 ml of pre-warmed Thaw Medium 1 (**no Geneticin or Puromycin**).
2. Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight.
3. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Geneticin or Puromycin**), and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split.
4. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1M (**contains Geneticin and Puromycin**).

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1M (**contains Geneticin and Puromycin**) and transfer to a tube.
3. Spin down cells, resuspend cells in Growth Medium 1M (**contains Geneticin and Puromycin**) and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:6 to 1:10 weekly or twice a week.

   Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with higher ratio.

Cell Freezing

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1M and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796 or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for 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

Note: Because this cell line depends on the Serum Response Element for luciferase reporter expression, some luciferase is expressed with 10% serum in the medium. For the assay, cells are seeded in 10% serum medium to aid in attachment of the cells (Thaw Medium 1), and then the medium is changed to 0.5% FBS overnight to increase the sensitivity of the assay (Assay Medium 1B).

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

A. Dose response of SRE Reporter – HEK293 cells to CSF1 and IL-34

1. Harvest CSF1R SRE Reporter – HEK293 cells from culture in growth medium 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. Leave a few of the wells empty for use as a cell-free control.

   Incubate cells at 37°C in a CO2 incubator overnight.

2. The next morning, carefully remove the medium from wells. Add 75 µl of Assay Medium 1B (0.5% serum) to wells.

   Incubate the plate at 37°C in a CO2 incubator for 20 to 24 hours.

3. Make a serial dilution of CSF1 or IL-34 in assay medium at 4x the final concentration.
   Gently add compounds to wells in 25 µl of Assay Medium 1B. Cells can detach easily.
   Add 25 µl of assay medium to the unstimulated control wells.
   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

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

4. Prepare luciferase reagents 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.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction for each treatment concentration = average background-subtracted luminescence of stimulated wells / average background-subtracted luminescence of unstimulated control wells.

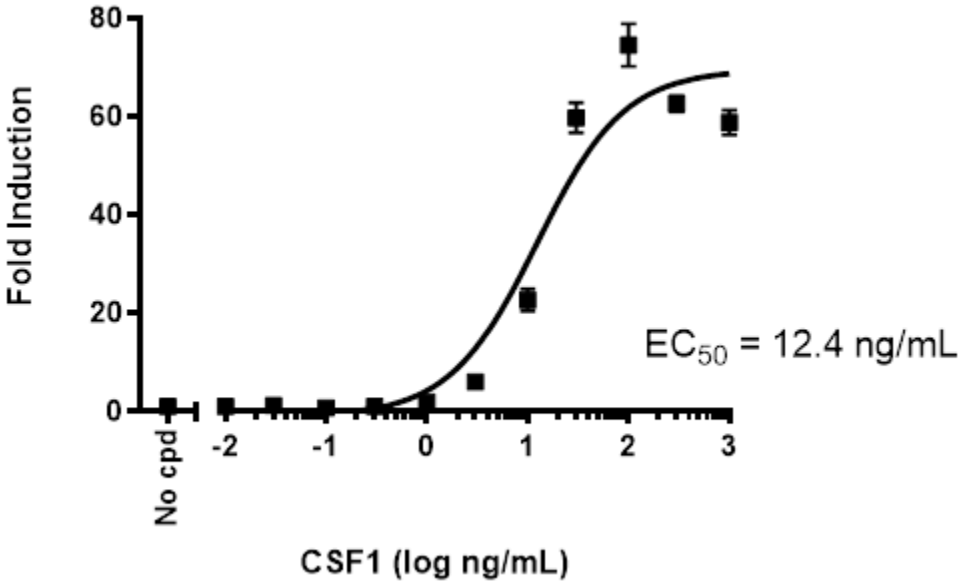

Figure 1a. Dose response of SRE reporter activity to CSF1 in the presence of 0.5% FBS. The results are shown as Fold Induction of SRE reporter activity.
The EC_{50} of CSF1 is ~12.4ng/mL.

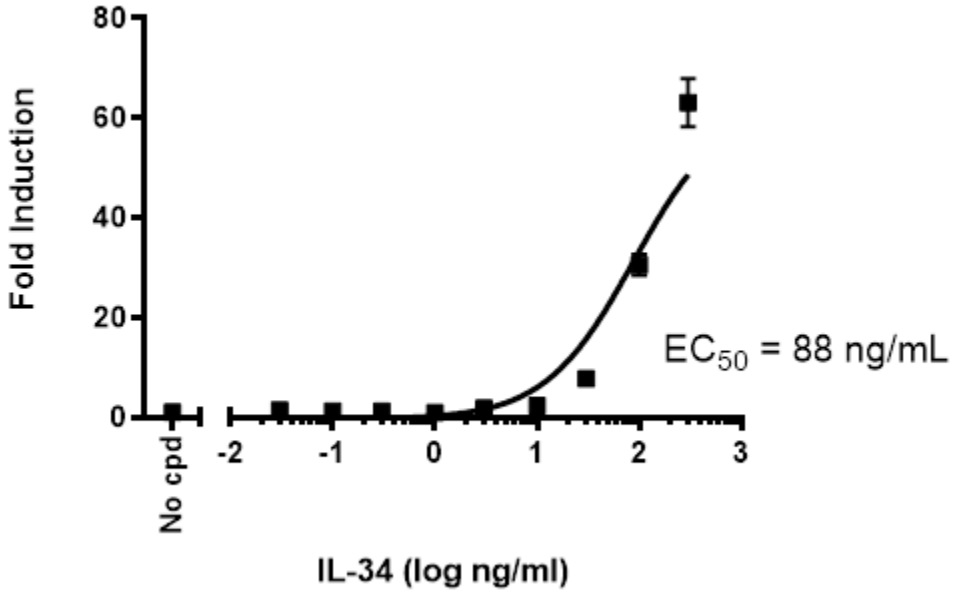

Figure 1b. Dose response of SRE reporter activity to IL-34 in the presence of 0.5% FBS. The results are shown as Fold Induction of SRE reporter activity.
The EC_{50} of IL-34 is ~88 ng/mL/

B. Inhibition of CSF1 or IL-34 induced reporter activity by an inhibitor of CSF1R in SRE Reporter – HEK293 cells

1. Harvest CSF1R SRE Reporter – HEK293 cells from culture in growth medium 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. Leave a few of the wells empty for use as a cell-free control.

   Incubate cells at 37°C in a CO_2 incubator overnight.

2. The next morning, carefully remove the medium from wells. Add 50 µl of Assay Medium 1B (0.5% serum) to wells.

   Incubate the plate at 37°C in a CO_2 incubator for 20 to 24 hours.

3. Make a serial dilution of CSF1R inhibitor in assay medium at 2.5x the final concentration. Maximum suggested DMSO concentration is 0.1%.

   Gently add compounds to wells in 40 µl of Assay Medium 1B. Cells can detach easily.
   Add 40 µl of assay medium to the unstimulated control wells.
   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.
   Incubate the plate at 37°C in a CO_2 incubator for 1 hour.

4. Add 10 µL of diluted CSF1 or IL-34 in assay medium to stimulated wells (final [CSF1] = 30 ng/ml) (final [IL-34] = 100 ng/ml). Add 10 µl of assay medium to the unstimulated control wells (cells without inhibitor and CSF1 or IL-34 treatment for determining the basal activity).

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

5. Prepare luciferase reagents using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 110 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.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of SRE luciferase reporter expression = background- subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells x 100%

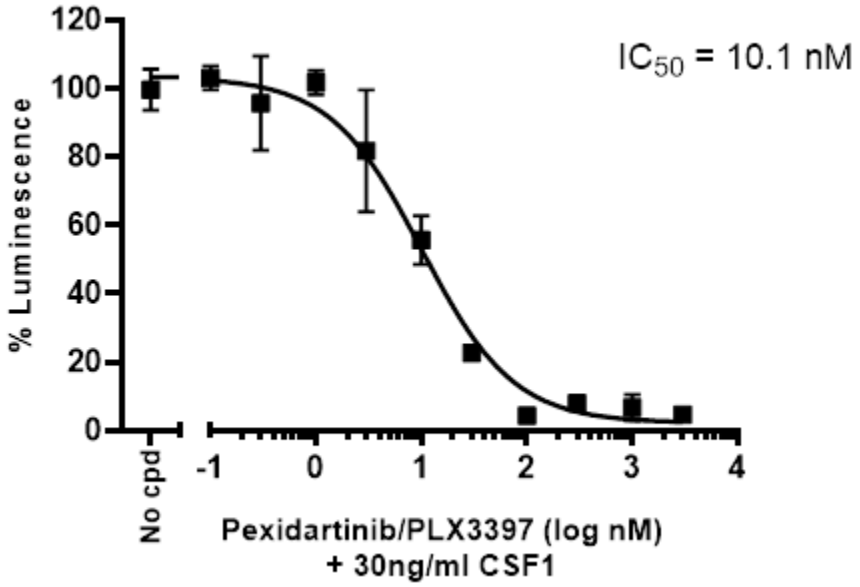

Figure 2a. Inhibition of CSF1-induced SRE reporter activity by CSF1R pathway inhibitor, PLX3397 (Pexidartnib).
The results are shown as percent luminescence of SRE reporter activity.
The IC_{50} of PLX3397 is 10.1 nM.

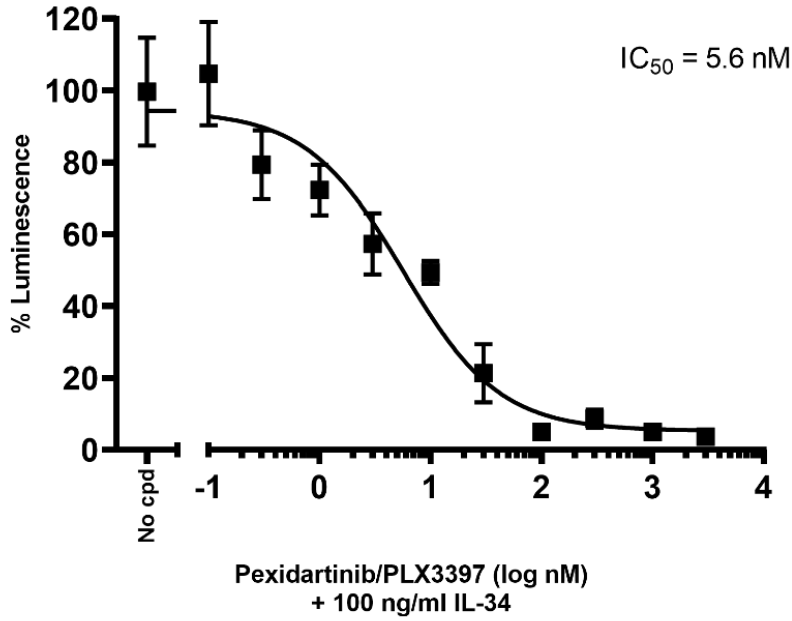

Figure 2b. Inhibition of IL-34-induced SRE reporter activity by CSF1R pathway inhibitor, PLX3397 (Pexidartnib).
The results are shown as percent luminescence of SRE reporter activity.
The IC_{50} of PLX3397 is 5.6 nM.

References

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2. Liu, Yang, and Xuetao Cao. 2014. "The Origin and Function of Tumor-Associated Macrophages." Cellular and Molecular Immunology, **12(1)**, 1–4., doi:10.1038/cmi.2014.83.
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4. Yao, G.-Q., et al. 2005. "CSF-1 Induces Fos Gene Transcription and Activates the Transcription Factor Elk-1 in Mature Osteoclasts." Calcified Tissue International, **76(5),** 371–378., doi:10.1007/s00223-004-0099-8.

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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.

Related Products

Products	*Catalog #*	*Size*
ONE-Step™ Luciferase Assay System	60690	10 ml/100 ml/1 L
Thaw Medium 1	60187	100 ml/500 ml
Growth Medium 1M	79723	500 ml
Assay Medium 1B	79617	100 ml/500 ml
SRE Reporter - HEK293 Recombinant Cell Line (ERK Pathway)	60406	2 vials
CSF1R / SRE Reporter Kit (MAPK/ERK Signaling Pathway)	79379	500 reactions
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 reactions