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Data Sheet
CSF1R / SRE – Reporter HEK293 Recombinant Cell Line
Catalog #: 79380

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.

Description

The CSF1R SRE 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.

Applications

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

Format
Two vials containing ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt. Do not store for long-term at -80°C or on dry ice.

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Mycoplasma Testing
This cell line has been screened using the Venor™ GeM Mycoplasma Detection Kit, PCR Based (Sigma, #MP0025) to confirm the absence of Mycoplasma contamination.

Culture Medium:

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% 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).

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

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

If culturing cells in medium from other vendors, it may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

Recommended Culture Condition:

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 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin or Puromycin)**.Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. 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. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1M **(contains Geneticin and Puromycin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1M **(contains Geneticin and Puromycin)** and transfer to a tube. 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 ration: 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 cell can be split with higher ratio.

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To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (no Geneticin or Puromycin) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~$2x10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

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 (BPS Bioscience, #60187)
- Growth Medium 1M (BPS Bioscience, #79723)

- **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).

- Recombinant human CSF1 (BPS Bioscience, #90215)
- 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

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). Use of coated plates may help decrease loss of cells when changing medium.

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Transfection and Assay Protocol – Ligand dose response

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 90 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 CSF1 or IL-34, controlling for the same amount of PBS in each dilution.

   Gently add compounds to wells in 10 µl of Assay Medium 1B. Cells can detach easily.
   Add 10 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 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.

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.

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Figure 1a. Dose response of SRE reporter activity to CSF1 in the presence of 0.5% FBS.
The results are shown as Relative Light Units of SRE reporter activity.
The EC50 of CSF1 is ~18.2 ng/mL.

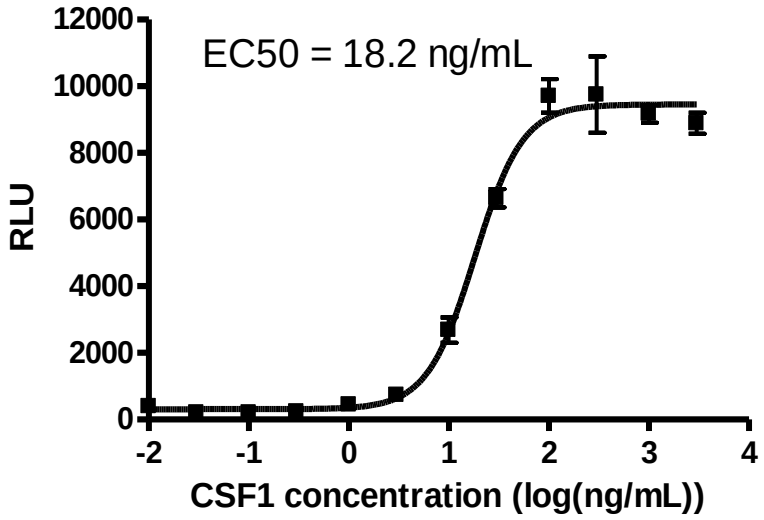
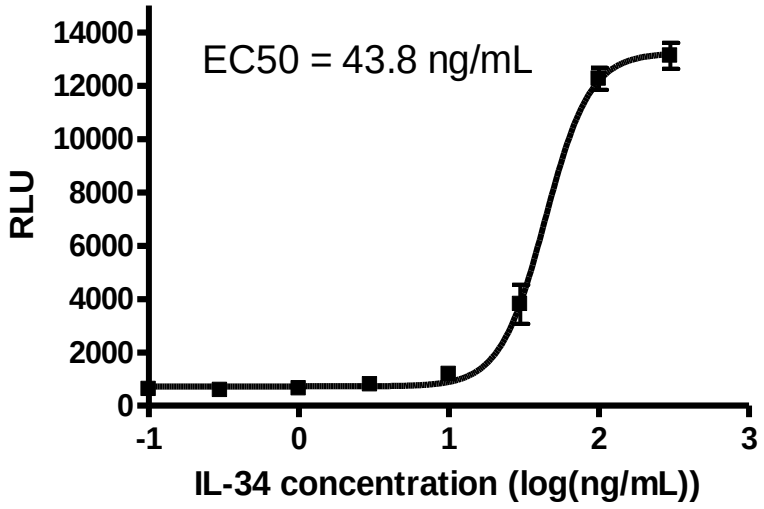


Figure 1b. Dose response of SRE reporter activity to IL-34 in the presence of 0.5% FBS.
The results are shown as Relative Light Units of SRE reporter activity.
The EC50 of IL-34 is ~43.8ng/mL.



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Transfection and Assay Protocols – CSF1R inhibitor

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, controlling for the same amount of DMSO in each dilution. Maximum suggested DMSO concentration is 0.1%.

   Gently add compounds to wells in 50 µl of Assay Medium 1B. Cells can detach easily.
   Add 50 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. After 1 hour, treat cells with CSF1 or IL-34 to activate CSF1R signaling. Add 10 µL Assay Medium 1B containing 110 ng/mL CSF1 or 495 ng/mL IL-34 to each well, for a final concentration of 10 ng/mL CSF1 or 45 ng/mL IL-34.
   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 fold induction for each treatment concentration = average background-subtracted luminescence of stimulated wells / average background-subtracted luminescence of unstimulated control wells.

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Figure 2a. Inhibition of CSF1-induced SRE reporter activity by CSF1R pathway inhibitor, PLX3397 (Pexidartnib). The results are shown as Relative Light Units of SRE reporter activity. The IC50 of PLX3397 is 18.2 nM.

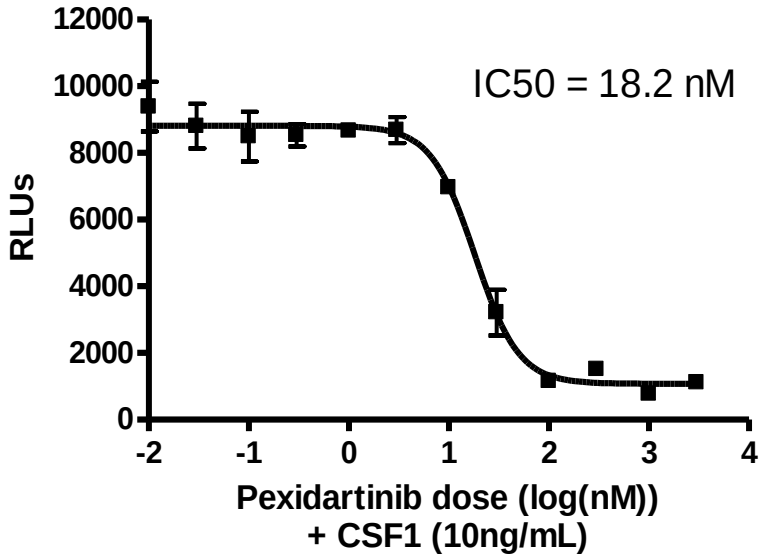
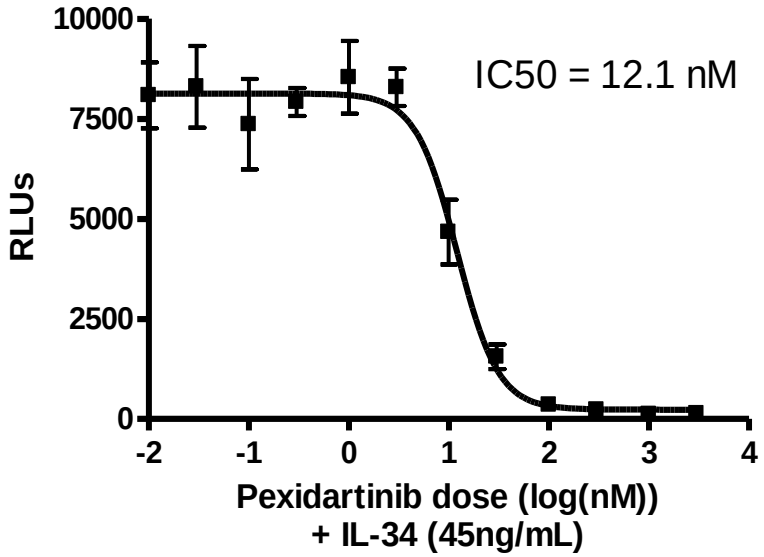


Figure 2b. Inhibition of IL-34-induced SRE reporter activity by CSF1R pathway inhibitor, PLX3397 (Pexidartnib). The results are shown as Relative Light Units of SRE reporter activity. The IC50 of PLX3397 is 12.1 nM.



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References

1. Cannarile, Michael A., *et al.* 2017. "Colony-Stimulating Factor 1 Receptor (CSF1R) Inhibitors in Cancer Therapy." Journal for Immunotherapy of Cancer, **5(1),**(53), doi:10.1186/s40425-017-0257-y.
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.
3. Luo, Jian, *et al.* 2013. "Colony-Stimulating Factor 1 Receptor (CSF1R) Signaling in Injured Neurons Facilitates Protection and Survival." The Journal of Experimental Medicine, **210(1),** 157–172., doi:10.1084/jem.20120412.
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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Related Products

Product Name	Catalog #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 mL
ONE-Step™ Luciferase Assay System	60690-2	100 mL
ONE-Step™ Luciferase Assay System	60690-3	1 L
Thaw Medium 1	60187-1	100 mL
Thaw Medium 1	60187-2	500 mL
Growth Medium 1M	79723	500 mL
Assay Medium 1B	79517-1	100 mL
Assay Medium 1B	79517-2	500 mL
SRE Reporter – HEK293 Cell Line	60406	2 vials
CSF1R SRE Reporter Kit	79379	500 reactions
SRE Reporter Kit	60511	500 reactions
Human Macrophage Colony Stimulating Factor	90215-A	2 µg
Human Macrophage Colony Stimulating Factor	90215-B	10 µg
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