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Data Sheet

PAI-1 Reporter (Luc) – Mv1 Lu Cell line

Catalog #: 60544

Description
PAI-1 Reporter (Luc)-Mv1 Lu cell line is designed for monitoring transforming growth factor β (TGF-β)-induced plasminogen activator inhibitor-1 (PAI-1) expression. Transforming growth factor-β (TGF-β) is a potent regulator of cellular differentiation, proliferation, migration, and protein expression.

PAI-1 Reporter (Luc) –Mv1 Lu cell line contains a firefly luciferase gene under the control of PAI-1 responsive elements stably integrated into Mv1 Lu (NBL-7) cells, showing TGF-β pathway response. This cell line is validated for the TGF-β response to the induction of PAI-1 gene expression through luciferase activity.

Application
- Monitor TGF-β signaling pathway activity

Format
Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Complete Growth Medium: Thaw Medium 1 (BPS Cat. #60187) and 700 µg/ml of Geneticin (Invitrogen, #11811031).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

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)**, and spin down the cells. Resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)** transfer the resuspended cells

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to a T25 flask, and culture in a 37°C CO_2 incubator. At first passage, switch to growth medium containing Geneticin. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.25% Trypsin/EDTA, add complete growth medium and transfer to a tube. Spin down cells, resuspend them, and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:10 to 1:20 weekly

Functional Validation and Assay Performance
The following assays are designed for a 96-well format. To perform the assay in other tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- Human TGF-β1 (R+D Systems #240-B-002,)
- Thaw Medium 1 (BPS Bioscience, #60187)
- Growth medium: Thaw Medium 1 + G418 700 µg/ml.
- Assay medium: DMEM medium (Hyclone, #SH30243.01) + 1% BSA + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-STEP™ Luciferase Assay System (BPS Bioscience, #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

A. Dose response of PAI-1 Reporter (Luc)-Mv1 Lu cells to TGF-β1

1. Harvest PAI-1 Reporter (Luc)-Mv1 Lu cells from culture in Growth medium and seed cells at a density of 20000~30,000 cells per well into white clear-bottom 96-well microplate in 100 µl of growth medium without selection (G418) at 37°C in a CO_2 incubator overnight. Leave a couple of wells empty for the cell free control.

2. Prepare TGF-β1 solution in assay medium (10-point serial dilutions from 100 ng/ml).

3. Remove the growth medium from cells and add 60 µl of diluted TGF-β1 to each test well. Add 60 µl of assay medium to the unstimulated control wells and 60 µl of assay medium to the cell-free control wells (for determining background luminescence). *Set up each treatment in at least triplicate.*

4. Incubate cell plates at 37°C in a CO_2 incubator for ~ 24 hours.

5. Perform luciferase assay using ONE-STEP™ luciferase assay system: Add 60 µl of One-STEP Luciferase reagent per well and rock gently at room temperature for 30 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

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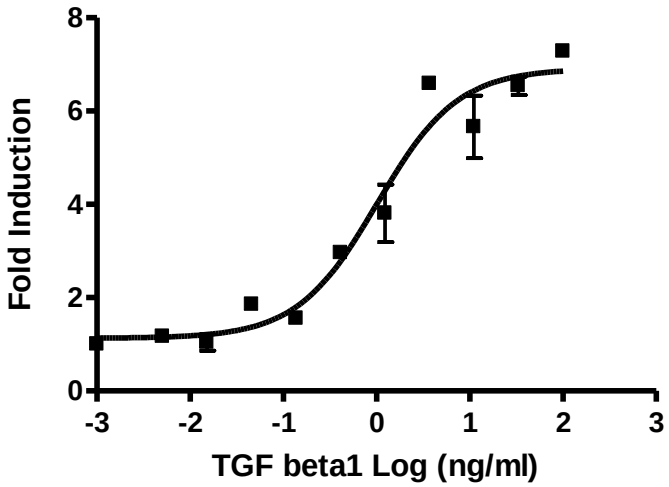
Fold induction of PAI-1 luciferase reporter expression = background-subtracted luminescence of TGF-β1-stimulated well / average background-subtracted luminescence of unstimulated control wells

Figure 1. Dose response of PAI-1 Reporter (Luc)-Mv1 Lu cells to human TGF-β1

The results are shown as fold induction of PAI-1 luciferase reporter expression.
The EC50 of TGF-β1 is ~ 1 ng/ml.

EC50= 1.018ng/ml



Sequence
PAI-1 promoter sequence (874bp-EcoR1/Hind III)

AAGCTTTTACCATGGTAACCCCTGGTCCCGTTCAGCCACCACCACCCCACCCAGCACACCT
CCAACCTCAGCCAGACAAGGTTGTTGACACAAGAGAGCCCTCAGGGGCACAGAGAGAGTC
TGGACACGTGGGGAGTCAGCCGTGTATCATCGGAGGCGGCCGGGCACATGGCAGGGATG
AGGGAAAGACCAAGAGTCCTCTGTTGGGCCCAAGTCCTAGACAGACAAAACCTAGACAAT
CACGTGGCTGGCTGCATGCCCTGTGGCTGTTGGGCTGGGCCCAGGAGGAGGGAGGGGC
GCTCTTTCCTGGAGGTGGTCCAGAGCACCGGGTGGACAGCCCTGGGGGAAAACTTCCAC
GTTTTGATGGAGGTTATCTTTGATAACTCCACAGTGACCTGGTTCGCCAAAGGAAAAGCAG
GCAACGTGAGCTGTTTTTTTTTTCTCCAAGCTGAACACTAGGGGTCCTAGGCTTTTTGGGT
CACCCGGCATGGCAGACAGTCAACCTGGCAGGACATCCGGGAGAGACAGACACAGGCAG
AGGGCAGAAAGGTCAAGGGAGGTTCTCAGGCCAAGGCTATTGGGGTTTGCTCAATTGTTC
CTGAATGCTCTTACACACGTACACACACAGAGCAGCACACACACACACACACACATGCCTC
AGCAAGTCCCAGAGAGGGAGGTGTCGAGGGGGACCCGCTGGCTGTTCAGACGGACTCCC
AGAGCCAGTGAGTGGGTGGGGCTGGAACATGAGTTCATCTATTTCCTGCCCACATCTGGT
ATAAAAGGAGGCAGTGGCCCACAGAGGAGCACAGCTGTGTTTGGCTGCAGGGCCAAGAG
CGCTGTCAAGAAGACCCACACGCCCCCCTCCAGCAGCTGAATTC

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References

Abe, M. *et al*. (1994) An assay for transforming growth factor- β using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. *Analytical Biochemistry* **216:**276-584.

Khan, S. *et al*. (2012) Quantification of active and total transforming growth factor-β levels in serum and solid organ tissues by bioassay. *BMC Research Notes* **5:** 636

Related Products

Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
TGFβ1	90900-1	1 µg
TGFβ1	90900-2	5 µg
TGFβ1	90900-10	10 µg
TGFβ1	90900-3	1 mg
TGFβ1, Latent	90901-1	5 µg
TGFβ1, Latent	90901-2	25 µg
TGFβ1, Latent	90901-3	1 mg
TGFβR2, GST-tag	40707	50 µg
TGF/SMAD Signaling Pathway SBE Reporter – HEK293 Cell Line	60653	2 vials

Notes

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