

## RPMI 8226/Luc Stable Cell Line Development Service Data Sheet

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## RPMI 8226/Luc Stable Cell Line

Catalog No.	Size
SCRPM-STP320	2 × (1 vial contains ~5×10 <sup>6</sup> cells)

### • Description

The RPMI 8226/Luc Stable Cell Line was engineered to express the firefly luciferase reporter. The luciferase activity was confirmed by the detection of luminescence signal.

### • Application

- Used as target cells for luminescence-based cytotoxicity evaluation.

### • Cell Line Profile

Cell line	RPMI 8226/Luc Stable Cell Line
Host Cell	RPMI 8226
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS + L-Glutamine (2 mM)
Selection Marker	Puromycin (0.5 µg/mL)
Incubation	37°C with 5% CO <sub>2</sub>
Doubling Time	20-30 hours
Transduction Technique	Lentivirus

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## • *Materials Required for Cell Culture*

- RPMI Medium 1640 (ATCC, Cat. No. 30-2001)
- Fetal bovine serum (Gibco, Cat. No. A5669701)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)

**Note:** For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- L-Glutamine (Gibco, Cat. No. 25030081)
- Complete Growth Medium: RPMI-1640 + 10% FBS, 1%P/S, L-Glutamine (2 mM)
- Culture Medium: RPMI-1640 + 10% FBS, Puromycin (0.5 µg/mL), 1%P/S, L-Glutamine (2 mM)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO<sub>2</sub> Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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## • *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium.
4. Count viable cells and centrifuge at approximately 1000 rpm for 5 minutes.
5. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh **complete growth medium**. Adjust the cell density of the suspension to  $1 \times 10^6$  viable cells/mL and transfer cells to an appropriate size vessel.
6. Incubate at 37°C with 5% CO<sub>2</sub> incubator.

## • *Subculture*

Cell viability may be low after thawing, and full recovery (viability >90%) may take up to 1-2 weeks. Once the cell density reaches approximately  $1.5 \times 10^6$  viable cells/mL, adjust the density to a range of  $3 \times 10^5$ - $4 \times 10^5$  viable cells/mL by either adding the fresh **culture medium** or replacing the existing culture medium. Avoid allowing the cell density to exceed  $2 \times 10^6$  cells/mL, as this may negatively impact cell performance in subsequent passages. T-75 flasks are recommended for subculturing.

• **Subculturing Frequency:** It is recommended to subculture every 3-4 days, adjusting the frequency based on the cell density in your specific culture system.

**Note:** After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition (viability >90%), transition to the culture medium containing the selection marker during subculturing.

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## • *Cryopreservation*

1. Count viable cells and harvest the cell suspension.
2. Centrifuge at 1000 rpm for 5 min at room temperature and resuspend cells in ice cold freezing medium to a concentration of  $5 \times 10^6$  to  $1 \times 10^7$  cells/mL.
3. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a  $-80^\circ\text{C}$  freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

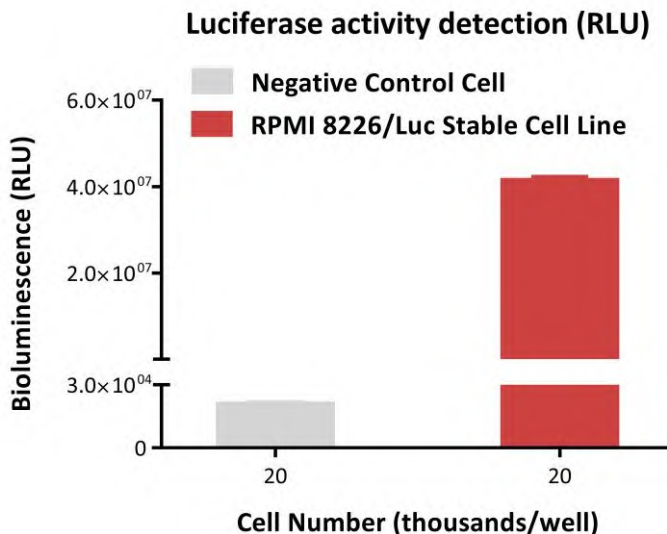
**Note:** It is recommended to establish a cell bank at the earliest possible passage for long-term use.

## • *Storage*

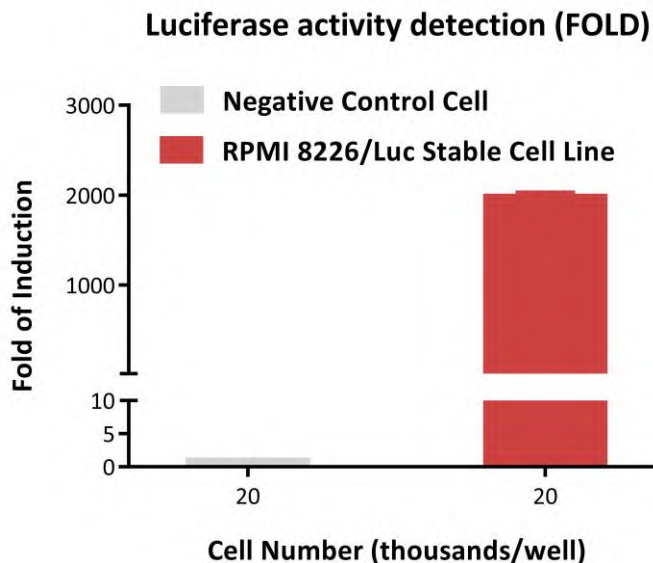
Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a  $-80^\circ\text{C}$  freezer immediately upon receipt. If stored in a  $-80^\circ\text{C}$  freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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• *Luminescence Assay*



**Fig1. Expression analysis of luciferase on RPMI 8226/Luc Stable Cell Line (RLU).** The luminescence signal of RPMI 8226/Luc Stable Cell Line were detected by incubating with the luciferase substrate. The RLU value was approximately  $4.14 \times 10^7$  at the density of  $2 \times 10^4$  cells/well.



**Fig2. Expression analysis of luciferase on RPMI 8226/Luc Stable Cell Line (FOLD).** The luminescence signal of RPMI 8226/Luc Stable Cell Line were detected by incubating with the luciferase substrate. The max induction fold was approximately 1984.9 at the density of  $2 \times 10^4$  cells/well.

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## • *Related Products*

<u>Products</u>	<u>Cat. No.</u>
Human 4-1BB (Luc) HEK293 Reporter Cell	CHEK-ATF073
CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	SCCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	SCCHO-ATP059M
CHO/Human CD16a (158V) Stable Cell Line (High Expression)	SCCHO-ATP059H
CHO/Human CD32b Stable Cell Line (Low Expression)	SCCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression)	SCCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression)	SCCHO-ATP060H
CHO/Human CD32a Stable Cell Line (Low Expression)	SCCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression)	SCCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression)	SCCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression)	SCCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression)	SCCHO-ATP062M
CHO/Human CD64 Stable Cell Line (High Expression)	SCCHO-ATP062H
CHO/Human PD-L1 Stable Cell Line (Low Expression)	SCCHO-ATP077L
CHO/Human PD-L1 Stable Cell Line (Medium Expression)	SCCHO-ATP077M
CHO/Human PD-L1 Stable Cell Line (High Expression)	SCCHO-ATP077H
CHO/Human CD32a (131R) Stable Cell Line	SCCHO-ATP223
CHO/Human CD16a (158F) Stable Cell Line	SCCHO-ATP224
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044
NF- $\kappa$ B (Luc) HEK293 Reporter Cell	CHEK-ATF048
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080

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## • *Related Products*

<u>Products</u>	<u>Cat. No.</u>
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
NY-ESO-1 specific TCR-HEK293 cell line	CHEK-STP114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
Human BMP (Luc) HEK293 Reporter Cell	CHEK-ATF188
HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP199
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200
HEK293/Human NPR1 Stable Cell Line	CHEK-ATP245
CHO/Human NPR1 Stable Cell Line	CCHO-ATP290