

**Description**

Recombinant CCRF-CEM cells constitutively expressing the firefly (*Photinus pyralis*) luciferase reporter gene under the control of a CMV promoter.

**Background**

CCRF-CEM is a human leukemia cell line derived from the peripheral blood of a patient with acute lymphoblastic leukemia. CCRF-CEM contains a somatic mutation that impairs the glucocorticoid receptor, which has been linked to acute lymphoblastic leukemia. The CCRF-CEM cell line provides a valuable model for studying possible roles that glucocorticoids play in leukemic cell growth. The signal generated by the firefly luciferase is proportional to cell numbers.

**Application(s)**

- Use as an internal control in CAR-T or NK co-culture killing assays
- *In vitro* and *in vivo* bioluminescence imaging

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains $2 \times 10^6$ cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

**Parental Cell Line**

CCRF-CEM, human T lymphoblasts derived from a patient with acute lymphoblastic leukemia, suspension

**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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

*Media Required for Cell Culture*

Name	Ordering Information
Thaw Medium 2	<a href="#">BPS Bioscience #60184</a>
Growth Medium 2D	<a href="#">BPS Bioscience #79639</a>

*Materials Required for Cellular Assay*

Name	Ordering Information
ONE-Step™ Luciferase Assay System	<a href="#">BPS Bioscience #60690</a>
96-well Flat Clear Bottom White Polystyrene TC-treated Microplates	Corning #3610
Luminometer	

**Storage Conditions**

Cells are shipped in 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. Other preparations or formulations of media may result in suboptimal performance.



Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.

Cells should be grown at 37°C with 5% CO<sub>2</sub>. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

*Media Required for Cell Culture*

*Thaw Medium 2 (BPS Bioscience #60184):*

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

*Growth Medium 2D (BPS Bioscience #79639):*

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin B.

**Cell Culture Protocol***Cell Thawing*

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2 (**no Hygromycin**).

**Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Hygromycin**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator.
4. After 24 hours of culture, check for cell viability. For a T25 flask, add 2-3 ml of Thaw Medium 2 (**no Hygromycin**), and continue growing in a 5% CO<sub>2</sub> incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach a density of 1 x 10<sup>6</sup> cells/ml. At first passage and subsequent passages, use Growth Medium 2D (**contains Hygromycin**).

*Cell Passage*

Dilute the cell suspension into new culture vessels before they reach a density of 1 x 10<sup>6</sup> cells/ml, at no less than 0.2 x 10<sup>6</sup> cells/ml of Growth Medium 2D (**contains Hygromycin**). The sub-cultivation ratio should maintain the cells between 0.2 x 10<sup>6</sup> cells/ml and 1 x 10<sup>6</sup> cells/ml.

### Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of  $\sim 2 \times 10^6$  cells/ml.
2. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
3. Transfer the vials to liquid nitrogen the next day for storage.



Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

### Validation Data

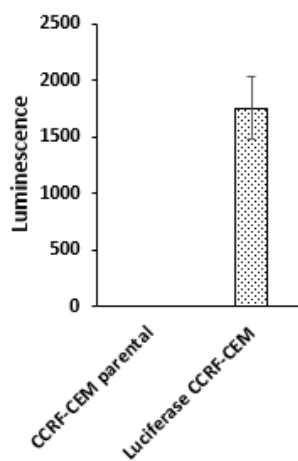


Figure 1: Luciferase activity in Firefly Luciferase CCRF-CEM recombinant cells.

Firefly Luciferase CCRF-CEM recombinant cells were seeded into a 96-well plate at  $2.35 \times 10^4$  cells/well in 100  $\mu$ l Thaw Medium 2 (BPS Bioscience #60184), and the luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience #60690).

### Reference

Lambrou, G., et al. *International Journal of Molecular Sciences*, **22(11)**: 5889.

### License Disclosure

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### Troubleshooting Guide

Visit [bpsbioscience.com/cell-line-faq](https://bpsbioscience.com/cell-line-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase Lentivirus	79692	2 vials
Firefly Luciferase-eGFP Lentivirus	79980	2 vials
Firefly Luciferase KG-1 Cell Line	78493	2 vials
Firefly Luciferase NALM6 Cell Line	78494	2 vials
Firefly Luciferase NK-92 Cell Line	78400	2 vials
Firefly Luciferase SKOV-3 Cell line	78425	2 vials