Research Use Only. Not for use in diagnostic procedures.

Bioware[®] Brite Cell Line LL/2 Red-FLuc Product No.: BW119267

Material Provided

Cells: 2 x 1 mL frozen aliquots (BW119267V)

Format: 1.0×10^6 cells / mL in 95% FBS, 5% DMSO

DESIGNATION	LL/2 Red-FLuc
Tissue	Mouse: lung carcinoma
Source of Parental Line	ATCC (CRL-1642)
Gene Transfer Vehicle	Red-FLuc-Puro 3d generation lentivirus
Bioluminescence In Vitro	At least 5,000 photons/cell/sec. Exact number will vary depending on imaging and culturing conditions.
Culture Properties	Mixed, adherent and suspension*; viability cannot be determined solely by cell attachment. Refer to the cell culture guidelines for more detailed instructions.
Recommended Media and FBS	DMEM ATCC Cat. No. 30-2002. Supplement the above with 10% Hyclone Fetal Bovine Serum (FBS) GE HealthCare Cat. No. 300071.
Recommended Storage Conditions	Remove frozen cells from dry ice packaging and immediately place cells at a temperature below -130° C, preferably in liquid nitrogen vapor, until ready to use.
Average Doubling Time	24 hours
Other Recommendations	When initially thawing, use T25 flask or 10cm plate. Cells should be ready to expand within 1-4 days. Antibiotics can be used in the media if desired after the initial thaw. (puromycin at 2ug/mL). Refer to Cell Culture Guidelines for more detailed instructions.

* Please refer to Morphology on page 2 of this document.

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The Features

Perkin Elmer Bioware® Brite cell line models offer researchers the ability to:

- Monitor early tumor development
- Monitor tumor growth and metastases in vivo
- Quantify tumor burden in the whole animal
- Follow responses to therapeutic treatments non-invasively in longitudinal studies using the same cohorts of mice

Murine Pathogen Free

All Perkin Elmer cell lines are confirmed to be pathogen free by the IMPACT Profile I (PCR) at the University of Missouri Research Animal Diagnostic and Investigative Laboratory.

Cell Line Stability

Cell may undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is recommended to prepare an adequate number of frozen stock at early passages.

Product Warranty

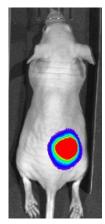
PerkinElmer warrants that cells will be viable upon shipment from PerkinElmer for a period of thirty days, provided they have been properly stored and handled during this period.

Murine Lung Carcinoma Cell Line: LL/2 Red-FLuc

LL/2 Red-FLuc is a luciferase expressing cell line which was stably transfected with firefly luciferase gene from *Luciola Italica* (Red-FLuc). The cell line was established by transducing lentivirus containing Red-FLuc luciferase under the control of human ubiquitin C promoter. These cells will serve as a new tool to detect drug efficacy *in vitro* and *in vivo* with high sensitivity.

Morphology

LL/2 Red-FLuc is a mixture of adherent and suspension cells that will normally appear in culture as rounded and loosely attached or fully suspended cells. Expect to see irregularly shaped clusters of cells in suspension for the first several days. Cells that do attach may resemble epithelial morphology, but can detach easily and form large suspended aggregates of healthy, growing cells. Refer to Cell Culture Guidelines for more detailed instructions.



Bioluminescence image of LL/2 Red-FLuc subcutaneous tumor

Growth Curve of LL/2 Red-FLuc Cells

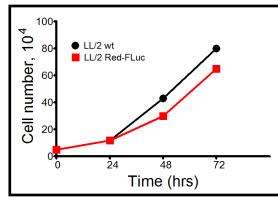


Figure 1. 5×10^4 cells were plated on a 6cm plate. The total numbers of cells were counted every 24 h using a Nexcelom automatic cell counter.

In Vitro BLI Signal Stability

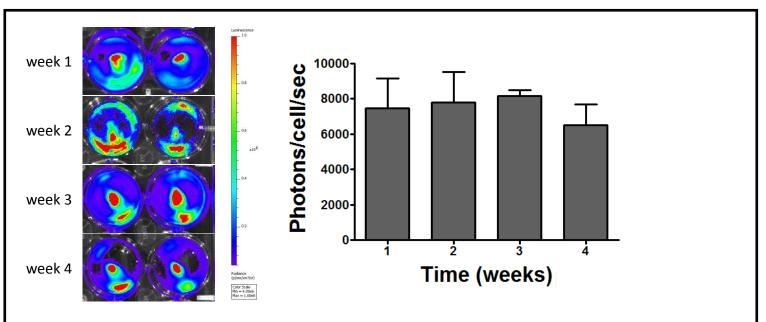


Figure 2. 5×10^4 cells were plated per well in 24-well plates. Cells were incubated at 37 °C for recovery overnight and luciferase assay was performed using the PerkinElmer IVIS[®] SpectrumCT. Each experiment was done in quadruplicates. The cells were maintained in continuous culture over four weeks and weekly luciferase assay was performed. Bioluminescence data was analyzed using the Living Image 4.0 software.

Subcutaneous Tumor Growth in a Nu/nu Mouse

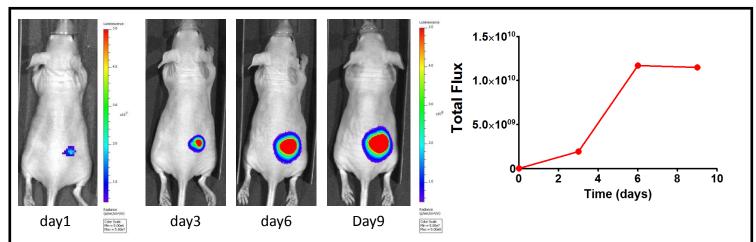


Figure 3. $1 \ge 10^6$ LL/2 Red-FLuc cells were injected subcutaneously into the dorsal region near the thigh of female nu/nu mouse. Tumor growth was monitored for luciferase expression using the PerkinElmer IVIS[®] Spectrum at various time points. Mice were imaged 10 minutes post i.p. injection of luciferin at 150mg/kg at various time points. The image above shows tumor growth from a representative mouse.

Tumor Growth Comparison Between Wild Type and Red-FLuc Cells

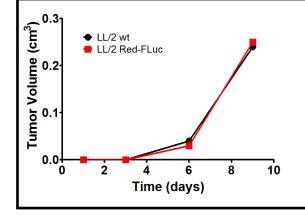


Figure 4. $1 \ge 10^6$ LL/2 Red-FLuc and LL/2 parental cells were injected subcutaneously into the dorsal region near the thigh of female nu/nu mouse. Tumor growth was monitored by caliper measurements at various time points. Similar tumor growth rate was observed for both parental and Red-FLuc transduced cell lines.

For more information on our in vivo imaging agents, please visit our website: www.perkinelmer.com/bioware.

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