Bioware® Brite Light-Producing Cancer Cell Lines and Substrates

Caution: For Research Use. This product is intended for animal research only and not for use in humans. Not for human or animal therapeutic or diagnostic use.

General FAQ:

1. What is the gene transfer vehicle for expressing luciferase in our Bioware® Brite Cell Lines ?

We use RediFect Red-Fluc-Puromycin (PerkinElmer Product Number CLS960002, puromycin as selection marker) 3d generation lentivirus or Red-Fluc-GFP (PerkinElmer Product Number CLS960003) 3d generation lentivirus as gene transfer vehicles for Bioware® Brite cell lines.

2. What types of oncology models can be generated by using Bioware® Brite cell lines ?

Currently PerkinElmer offers cancer cell lines covering a range of oncology *in vivo* models including cancer of the breast, blood (Leukemia), brain, colorectal tract, fibrosarcoma, liver, lung, skin (melanoma), ovary, pancreas, and prostate. These cells can be used for either *in vitro* or *in vivo* research.

Please refer the links below for details of light-producing cancer cell lines:

http://www.perkinelmer.com/Catalog/Category/ID/Cell%20Lines

http://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/in-vivo-preclinical-imaging/in-vivo-preclinical-imaging-main.html

3. Is each Bioware® Brite cell line derived from a single clone or from a heterogeneous population of transfected luminescent cells?

Each of our Bioware® Brite cell linesis derived from a single cell clone. Clones are selected based upon their growth pattern (similar to the parent line), *in vitro* and *in vivo* bioluminescence properties (comparable growth kinetics, stability of luciferase expression,

appropriate brightness upon luciferin injection) and morphology in culture (similar to the parent line).

4. Have Bioware® Brite cell lines been screened to confirm that they are not contaminated?

Yes. All the Bioware® Brite cell lines have been screened for contamination by IMPACT I PCR evaluation profile, which includes testing for mycoplasma spp., mycoplasma pulmonis, mouse hepatitis virus, mouse minute virus, mouse parvovirus, Theiler's murine encephalomyelitis virus, Sendai virus, mouse pneumonia virus, mouse norovirus, reovirus 3, mouse rotavirus, ectromelia virus, lymphocytic choriomeningitis virus, polyomavirus, lactate dehydrogenase-elevating virus, mouse adenovirus, mouse cytomegalovirus, K virus, mouse thymic virus, and Hantaan virus.
All test results were negative.

5. Can all the Bioware® Brite cell lines be utilized for *in vitro* studies as well as *in vivo* studies?

Yes, all Bioware® Brite cell lines can be imaged *in vitro* by using an IVIS system, luminometer, bioluminescent plate reader or bioluminescence microscope. Please refer our citation library by searching bioluminescence: http://citations.perkinelmer.com/

6. What is the solubility of XenoLight D-Luciferin K+ salt (Product Number 122799) for *in vivo* studies?

XenoLight D-Luciferin K+ salt is offered as a solid white powder. It is soluble in H_2O and Phosphate Buffered Saline (up to 40 mg/ml concentration).

We also offer ready-to-use, pre-formulated, injectable Luciferin: RediJect (Product number 770504) and RediJect Ultra with a fluorescent tracer (770505) both in Phosphate Buffered Saline solution, 30mg/ml concentration.

7. What is the shelf life of XenoLight D-Luciferin K+ salt?

An unopened vial of lyophilized luciferin (122799) has a shelf life of approximately 2 years, if stored at -20°C and protected from light.

8. What are the optimal storage conditions for reconstituted XenoLight D-Luciferin K+ salt?

Ideally, a fresh stock solution is stored for a short period of time at -20°C, and we recommend a working solution is used immediate use after dilution. If necessary, luciferin solutions may be stored at 4°C or -20°C for up to 3 weeks. However, prolonged storage at either temperature may result in degradation of signal.

9. How can I determine the peak signal time of luciferin for different models?

The kinetics of bioluminescence signal can be tissue-dependent. We recommend creating a kinetic curve for each new model by imaging the animal every 5-10 minutes, up to 40 minutes. (For most models, the peak time point is around 10 to 15minutes).

Determining the Luciferin Kinetic Curve for Your Model:
http://www.perkinelmer.com/lab-solutions//resources/docs/TCH_Luciferin_Kinetic.pdf

Preparation of Luciferin for *In Vitro* and *In Vivo* Bioluminescent Assays:
http://www.perkinelmer.com/lab-solutions//resources/docs/TCH_Luciferin_Preparation.pdf

Basic Cell Culture FAQ

1. How should I store Bioware® Brite cells after arrival?

We ship the cells in vials packaged on dry ice to maintain the temperature during shipment. We suggest storing the vials immediately after arrival in liquid nitrogen freezers (lower than -130°C in vapor phase) as cryogenic sample storage.

2. What kind of incubator and cell culture medium can I use for Bioware® Brite cell lines?

All Bioware® Brite cell lines can be incubated at 37°C in 5% CO2 cell culture incubator. Please check each product data sheet or the table below for appropriate media composition.

3. How do I start a cell culture from a frozen vial of Bioware® Brite cells?

Before beginning any cell culture, please refer to our Bioware® Brite Cell Culture Guidelines available on our website, or a printed version will ship directly to you with your order.

Rapidly thaw the vial at water bath (37°C, 1-2 minutes), spray the surface of the vial with 70% (v/v) ethanol and move the vial into the cell culture hood for the procedures (please follow standard aseptic techniques).

It is important to minimize the time that cells are sitting in suspension containing DMSO, so to dilute DMSO, immediately transfer the thawed cells into a T25 flask containing 10 mL of culture medium and incubate at 37°C, 5-6% CO2, 100% humidity overnight.

Check the cell morphology and viability after 24 hours culture, change the medium as needed.

Flasks	Growth area (cm^2)	Recommended working volume (mL)	Recommended working stage
T-25	25	5 to 10	Initiation
T-75	75	15 to 25	Initiation or Passage
T-150	150	30 to 50	Passage
T-175	175	35 to 60	Expansion for *in vivo* implantation
T-225	225	45 to 75	Expansion for *in vivo* implantation

4. What is the average doubling time for Bioware® Brite cell lines?

The average *in vitro* doubling time varies from 14 - 60 hours, depending on the cell line. Please check the tables below for each cell line's average doubling time and media composition.

Bioware® Brite Cell Line Description	Product #	Media Composition	Average Doubling Time (h)
K562 Red-FLuc	BW124735	RPMI+10% FBS	**14**
U87 MG-Red-Fluc	BW124577	RPMI+10% FBS	**14**
GL261 Red-Fluc	BW134246	RPMI+10% FBS	**15**
MCF7 Red-Fluc	BW119262	EMEM+10% FBS	**20**

4T1 Red-Fluc	BW124087	EMEM+10% FBS	**24**
MDA-MB-231 Red-Fluc	BW124319	EMEM+10% FBS	**24**
4T1 Red-Fluc-GFP	BW128090	RPMI+10% FBS	**16**
MDA-MB-231 Red-Fluc-GFP	BW128442	RPMI+10% FBS	**60**
Colo205 Red-Fluc	BW124317	EMEM+10% FBS	**30**
HCT116 Red-Fluc	BW124318	EMEM+10% FBS	**34**
HT29 Red-Fluc	BW124353	DMEM+10% FBS	**26**
HT1080 Red-Fluc	BW128092	EMEM+10% FBS	**22**
HepG2 Red-Fluc	BW134280	EMEM+10% FBS	**24**
A549 Red-Fluc	BW119266	EMEM+10% FBS	**24**
LL/2 Red-Fluc	BW119267	RPMI+10% FBS	**36**
NCI-H460 Red-Fluc	BW124316	McCoy's 5a +10% FBS	**24**
B16F10 Red-Fluc	BW124734	McCoy's 5a +10% FBS	**16**
SKOV3 Red-Fluc	BW119276	RPMI+10% FBS	**15**
BxPC3 Red-Fluc	BW125058	RPMI+10% FBS	**28**
LNCaP Red-Fluc	BW125055	DMEM+10% FBS	**24**
PC3 Red-Fluc-GFP	BW133416	McCoy's 5a +10% FBS	**35**
PC3 Red-Fluc	BW128444	RPMI+10% FBS	**22**

5. How should I maintain my cell subculture?

In order to maintain the cells at the exponential growth phase, passages should be performed on a regular schedule. Assuming the cells exhibit normal morphology, they should be passaged once they reach 80% to 90% confluency in the flask. The correct split-ratio will depend on the cell doubling time and the purpose of your study. Cells should be fed regularly.

For *in vivo* study, we recommend harvesting the cells after less than 10 *in vitro* passages from the original vial. However, cells should be split at least one time before harvesting, which should be done during exponential growth when the cells achieve 80% to 90% confluency.

6. How do I generate my own cell stocks?

It is important to generate your own working supply of Bioware® Brite cells by freezing aliquots during the initial culture. When the cells have reached 80% confluence, aliquot in freezing media [5% DMSO/95% FBS without antibiotics] into cryogenic storage vials. Allow 10-20 minutes for the DMSO to penetrate the cells, then place the cryovials in an insulated freezer box at -80ºC. After 24 hours, transfer frozen vials to liquid nitrogen storage. After a day or two, retrieve one vial for thawing and culture to ensure that the batch of frozen cells retained proper viability.

Research & Troubleshooting FAQ

1. How do I prepare luciferin for *in vitro* study with cultured Bioware® Brite cells?

Materials needed:

XenoLight D-Luciferin, potassium salt
Sterile water
Complete media

Procedure:

A. Prepare a 200X luciferin stock solution (30 mg/ml) in sterile water. Mix gently by inversion until luciferin is completely dissolved. Use immediately, or aliquot and freeze at -20°C.

Note: One can either reconstitute the entire 1.0 g of D-Luciferin in 33.3 ml of sterile water to make the 30 mg/ml (200x) stock solution, or reconstitute the quantity of D-Luciferin necessary for an individual experiment.
Ideally, a stock solution is stored for a short period of time at -20°C, and we recommend a working solution is used immediate use after dilution. If necessary, luciferin solutions may be stored at 4°C or -20°C for up to 3 weeks. However, prolonged storage at either temperature may result in degradation of signal.

B. Prepare a 150 ug/ml working solution of D-Luciferin in pre-warmed tissue culture medium.

- Quick thaw 200X stock solution of luciferin and dilute 1:200 in complete media (150 ug/ml final).

C. Aspirate media from cultured cells.
D. Add 1x luciferin solution to cells just prior to imaging on your IVIS® Spectrum or Lumina imaging system.

Note: Incubating the cells for a short time at 37°C before imaging can increase the signal.

2. How do I use Bioware® Brite cells for *in vivo* study?

All human and mouse Bioware® Brite tumor cell lines can be injected into immunodeficient nu/nu mice to achieve tumor growth *in vivo*. The tumor cell lines of mouse origin can also be injected into normal immunocompetent mice, as long as they are of the same mouse strain as the tumor origin. Specific information regarding cell numbers and growth kinetics *in vivo* is available on the technical data sheet for each cell line. Subcutaneous, orthotopic, and metastatic models may be possible based on the origin and characteristics of the cell line.

Bioluminescent signal can be monitored throughout the experiment by systemic injection of luciferin prepared according to our protocol* and subsequent imaging on IVIS® Spectrum or Lumina imaging systems.

*Available on our website:
http://www.perkinelmer.com/labsolutions//resources/docs/TCH_Luciferin_Preparation.pdf

3. I made my own luciferase-expressing cell line using your RediFect Red-FLuc product, and the bioluminescence is decreasing. Why did this happen?

If your tumor line is not clonal, it is possible that non-Red-FLuc expressing tumor cells may be outgrowing the expressing tumor cells. Over time, this may lead to overall loss of signal.

If you have cloned your tumor cell line, you may have selected a clone that does not have stable expression of luciferase. A certain number of isolated clones may lose expression, so we routinely screen these clones for expression stability to identify the appropriate cell for commercialization.

4. Why aren't my Bioware® Brite cells growing in culture?

There are a variety of reasons why cells (either the original vials or your secondary stocks) may not be growing properly. The most commonly reported reasons include:

- Inadvertent thawing during shipping
- Not properly following the thawing and culturing procedures
- Improper freezing protocols, including:
  - Incorrect DMSO concentration
  - Freezing too rapidly
  - Not allowing DMSO to equilibrate into cells prior to freezing
  - Freezing at the wrong temperature
- Incorrect culture conditions

On only rare occasions is there a problem with PerkinElmer's Bioware® Brite stocks because of the rigorous Quality Control Processes used. If the above possibilities are unlikely, please contact PerkinElmer's technical support for more guidance.

5. Why aren't my Bioware® Brite cells growing well *in vivo*?

The success of tumor growth *in vivo* is greatly dependent on the characteristics of the cell line itself, how you maintain and propagate the line *in vitro*, how many cells you inject into the animals, and where you inject the cells.

Naturally, the best starting point is an understanding from the literature or from your own experience how the "wild-type" cell line performs *in vivo* and under what circumstances. In general, you should expect similar performance from your Bioware® Brite cell lines.

The most commonly reported reasons for poor *in vivo* performance include:

- Cell contamination
- Injecting overgrown cells
- Injecting too few cells for that particular line
- Using too small of a needle (<27g, which can shear the cell membranes)
- Injection into the wrong strain of mouse
- Mistakes in bioluminescence imaging

In addition, we have discovered that 4T1 and GL261 tumor cells that express our Red-FLuc can grow well in nu/nu mice but are immunologically rejected by normal immunocompetent

BALB/c recipients. We have *not* seen this same issue in other cell lines implanted in their syngeneic mouse strains.

If these possibilities are unlikely to be the cause of your problem, please contact PerkinElmer's technical support for more guidance.

6. Is there any way to image tumor biology changes in my Bioware® Brite cells *in vivo*?

It is possible to use multi-channel optical imaging to capture both tumor burden (by bioluminescence) as well as biological changes (measured by near infrared fluorescent agents) when imaging on the IVIS Spectrum or Lumina. PerkinElmer has a comprehensive portfolio of highly validated near infrared fluorescent imaging agents that can detect changes in tumor protease activity, cell death, cell receptors (αvβ3 integrin, folate, bombesin, Her2/neu, CAIX), and vascular/vascular leak. See our *In-Vivo* Imaging Agents brochure for further information or contact PerkinElmer's technical support for more guidance.

View or download our brochure at:
(http://www.perkinelmer.com/labsolutions//resources/docs/BRO_In_Vivo_Agents_Bioware_Brite.pdf)