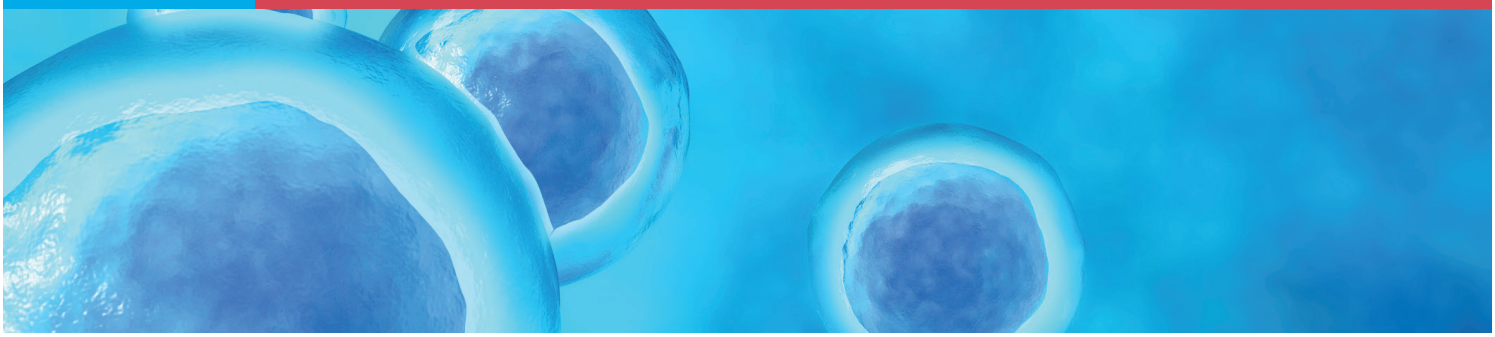
FREQUENTLY ASKED QUESTIONS

IVISbrite™ Tumor Cell Lines and Substrates



General FAQ

Q What is the gene transfer vehicle for expressing luciferase in our IVISbrite tumor cell lines?

A We use IVISbrite Red F-luc Puromycin (PerkinElmer Product Number CLS960002, puromycin as selection marker) 3rd generation lentivirus or Red F-luc-GFP (PerkinElmer Product Number CLS960003) 3rd generation lentivirus as gene transfer vehicles for IVISbrite cell lines.

Q What types of oncology models can be generated by using IVISbrite cell lines?

A Currently PerkinElmer offers tumor 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.

Q Is each IVISbrite cell line derived from a single clone or from a heterogeneous population of transfected luminescent cells?

A Each of our IVISbrite cell lines is 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).

Q Have IVISbrite cell lines been screened to confirm that they are not contaminated?

A Yes. All the IVISbrite 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.

Q Can all the IVISbrite cell lines be utilized for *in vitro* studies as well as *in vivo* studies?

A Yes, all IVISbrite cell lines can be imaged *in vitro* by using an IVIS® system, luminometer, bioluminescent plate reader or bioluminescence microscope.

Q What is the solubility of IVISbrite D-Luciferin Potassium Salt (Product Number 122799) for *in vivo* studies?

A IVISbrite D-Luciferin Potassium 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 D-Luciferin in a pre-formulated injectable format: IVISbrite D-Luciferin in RediJect™ Solution (Part number 770504) and IVISbrite D-Luciferin Ultra in RediJect Solution (Part number 770505) with a fluorescent tracer both in Phosphate Buffered Saline solution, 30 mg/ml concentration.

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Q What is the shelf life of IVISbrite D-Luciferin Potassium Salt?

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

Q What are the optimal storage conditions for reconstituted IVISbrite D-Luciferin Potassium Salt?

A 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 three weeks. However, prolonged storage at either temperature may result in degradation of signal.

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

A 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 15 minutes).

Please see 'Determining the Luciferin Kinetic Curve for Your Model' and 'Preparation of Luciferin for In Vitro and In Vivo Bioluminescent Assays', both available under Resources on the IVISbrite D-Luciferin product page of the PerkinElmer website.

Basic Tumor Cell Culture FAQ

Q How should I store IVISbrite tumor cells after arrival?

A 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.

Q What kind of incubator and cell culture medium can I use for IVISbrite tumor cell lines?

A All IVISbrite cell lines can be incubated at 37 °C in 5% CO_2 cell culture incubator. Please check each product data sheet or the table below for appropriate media composition.

Q How do I start a cell culture from a frozen vial of IVISbrite cells?

A *Before beginning cell culture, please refer to our* **IVISbrite Tumor Cell Culture Guidelines** *in combination with the individual Certificate of Analysis (Technical Data sheet) for each cell line before attempting any cell culture studies. The Cell Culture Guidelines and CoA are available on our website, or a printed version will ship directly 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 4 mL of culture medium and incubate at 37 °C, 5-6% CO_2, 100% humidity overnight. We recommend that you *do not* centrifuge the cells.

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

Q What is the average doubling time for IVISbrite tumor cell lines?

A 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.

Part Number	IVISbrite Tumor Cell Line Description	Media Composition*	Average Doubling Time*** (h)
BW124087	IVISbrite 4T1-Red-FLuc	RPMI+10% Hyclone FBS	14
BW128090	IVISbrite 4T1-Red-FLuc-GFP**	RPMI+10% Hyclone FBS	14
BW124734	IVISbrite B16F10-Red-FLuc	RPMI+10% Hyclone FBS	15
BW128444	IVISbrite PC3-Red-FLuc	EMEM+10% Hyclone FBS	24
BW133416	IVISbrite PC3-Red-FLuc-GFP**	EMEM+10% Hyclone FBS	24
BW124316	IVISbrite NCI-H460-Red-FLuc	RPMI+10% Hyclone FBS	16
BW125055	IVISbrite LNCaP-Red-FLuc	RPMI+10% Hyclone FBS	60
BW134280	IVISbrite HepG2-Red-FLuc	EMEM+10% Hyclone FBS	30
BW124577	IVISbrite U87MG-Red-FLuc	EMEM+10% Hyclone FBS	34
BW134246	IVISbrite GL261-Red-FLuc	DMEM+10% Hyclone FBS	26
BW128092	IVISbrite HT1080-Red-FLuc	EMEM+10% Hyclone FBS	22
BW125058	IVISbrite BxPC3-Red-FLuc	RPMI+10% Hyclone FBS	36
BW124353	IVISbrite HT-29-Red-FLuc	McCoy's 5a +10% Hyclone FBS	24
BW124318	IVISbrite HCT-116-Red-FLuc	McCoy's 5a +10% Hyclone FBS	16
BW124735	IVISbrite K562-Red-FLuc****	RPMI+10% Hyclone FBS	15
BW124317	IVISbrite Colo205-Red-FLuc****	RPMI+10% Hyclone FBS	28
BW119262	IVISbrite MCF7-Red-FLuc	EMEM+10% Hyclone FBS	40
BW119267	IVISbrite LL/2-Red-FLuc****	DMEM+10% Hyclone FBS	24
BW119276	IVISbrite SKOV3-Red-FLuc	McCoy's 5a +10% Hyclone FBS	35
BW119266	IVISbrite A549-Red-FLuc	RPMI+10% Hyclone FBS	22

* Optional: Puromycin at a final concentration of 2 ug/mL for all cell lines listed above, except for BW124087 which is at 5 ug/mL, and GFP cell lines which should not have any antibiotic added.

** GFP cell lines (BW128090 and BW133416) do not have any antibiotic selection resistance.

*** Doubling time is an average. Actual doubling times will vary based on culture conditions and handling.

**** Suspension cell lines

Q How should I maintain my cell subculture?

A 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.

Q How do I generate my own cell stocks?

A 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 -80°C. 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 and Troubleshooting FAQ

Q How do I prepare luciferin for *in vitro* study with cultured IVISbrite cells?

Materials needed:

- IVISbrite 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 three 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 imaging system.

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

Q How do I use IVISbrite tumor cells for *in vivo* study?

A All human and mouse IVISbrite tumor cell lines can be injected into immunodeficient nu/nu mice to achieve tumor growth *in vivo*. Some 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. (See the cell line technical data sheet for recommendation). 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 imaging systems.

Q I made my own luciferase-expressing cell line using your IVISbrite Red F-luc lentiviral particle product, and the bioluminescence is decreasing. Why did this happen?

A 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.

Q Why aren't my IVISbrite cells growing in culture?

A 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, such as:
  - Addition of antibiotic other than puromycin or antibiotic at the wrong concentration
  - Passaging too quickly/prior to confluence (cells will be diluted out, increasing doubling time and resulting in lower survival rates).

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

Q Why aren't my IVISbrite tumor cells growing well *in vivo?*

A 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 IVISbrite tumor 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 (<27 g, 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 F-luc 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.

Q Is there any way to image tumor biology changes in my IVISbrite tumor cells *in vivo*?

A 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 optical imaging platform. 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 (αvß3 integrin, folate, bombesin, CAIX), and vascular/vascular leak. See our *In Vivo* Imaging Agents brochure for further information or contact PerkinElmer's technical support for more guidance.

For more information on our IVISbrite tumor cell lines and other optical imaging reagents visit:
https://www.perkinelmer.com/category/in-vivo-imaging-reagents

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