



## Bioluminescent Reagents for In Vivo Optical Imaging

Bioluminescent imaging (BLI) is considered a modality of choice for high throughput imaging for a range of in vivo applications. Multiple animals can be imaged simultaneously with exposure times frequently in the range of seconds to minutes. Owing to excellent signal-to-noise ratios, BLI can provide sensitive imaging that is often leveraged for monitoring cell progression in tumor models, infection (viral, bacterial, and protozoan) models, and stem cell tracking studies. Firefly luciferase (FLuc) is used frequently for transfection/imaging with mammalian cells, but several variants exist including sources originating from luminescent beetles, bacteria, and marine species. FLuc requires an exogenous D-luciferin injection to produce luminescence, and is a necessary component of BLI experiments using firefly luciferase. Other luciferases require alternative substrates, or in the case of certain bacterial systems, substrates may be provided endogenously via co-expression constructs (Gahan CG, 2012; Xu et al., 2016; Kim et al., 2015).

Luciferase reporter genes may be readily introduced into cell lines of interest by transfection or transduction Luc vectors. There are now several commercial suppliers for Luc vector transfection/transduction kits. Additionally, there are commercial sources that offer Luc-cells. A partial list of such suppliers is shown in Table 1.

Pricing for bioluminescent imaging supplies can vary widely, and it can pay to shop around for the reporter variant, cell line, substrate and price to suit your experimental objectives. For example, a list of common tumor cell lines (transfected with FLuc or FLuc-iRFP reporters), Luc vectors, and substrates from three separate vendors and their respective pricing is shown in Table 2. Users of Bruker Optical/X-ray imaging systems have been successful using reagents from a range of suppliers. Figure 1 on the following page shows a BLI/X-ray rotation dataset acquired using the In-Vivo Xtreme II.

**Table 1. Suppliers of bioluminescent cells lines, transfection or transduction vectors, and substrates**

CAMBRIDGE BIOSCIENCE	<a href="http://www.bioscience.co.uk">http://www.bioscience.co.uk</a>
CLONTECH	<a href="https://www.clontech.com">https://www.clontech.com</a>
GOLDBIO	<a href="https://www.goldbio.com">https://www.goldbio.com</a>
IMANIS	<a href="https://www.imanislife.com">https://www.imanislife.com</a>
PERKIN ELMER	<a href="https://www.perkinelmer.com">https://www.perkinelmer.com</a>
PROMEGA	<a href="https://www.promega.com">https://www.promega.com</a>
SBI	<a href="https://www.systembio.com">https://www.systembio.com</a>
TARGETING SYSTEMS	<a href="http://www.targetingsystems.net">http://www.targetingsystems.net</a>

**Table 2. Bioluminescent reagent pricing can vary significantly between vendors and regions. Here average “academic” pricing in the USA was selected to compare different vendors. Reporter constructs available differ in the FLuc type and/or may include additional fluorescent reporters (e.g. iRFP).**

Reagent	Vendor 1	Vendor 2	Vendor 3
4T1	\$1,800 (FLuc-iRFP)	\$2,900 (FLuc)	N/A
HT1080	\$1,800 (FLuc-iRFP)	\$2,900 (FLuc)	N/A
B16F10	\$1,800 (FLuc-iRFP)	\$2,900 (FLuc)	N/A
CT26	\$1,800 (FLuc-iRFP)	N/A	N/A
A549	\$1,800 (FLuc-iRFP)	\$2,900 (FLuc)	N/A
Custom Cell Line	N/A	N/A	\$4000 (FLuc)
FLuc Vector	\$325-925 (FLuc)	N/A	\$750-2000 (FLuc)
Luciferin	N/A	\$260	\$175

**Figure 1**

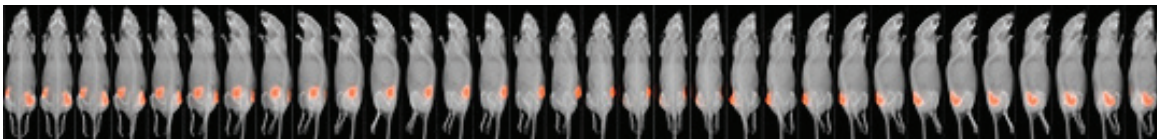


Figure 1. 360° Rotational Luminescent and X-Ray Imaging: Athymic nude mice (females, 4-5 weeks old) were challenged with human prostate tumor cells, previously transduced to express the Firefly luciferase reporter (PC3M cells from Targeting Systems, El Cajon, CA). Mice received bilateral, subcutaneous, dorsal, bolus injections (2 x 10<sup>6</sup> cells/100 µL 1xPBS). At 8 days post-cell challenge, PC3M tumor size and location was evaluated non-invasively by acquiring tumor luminescent signals, overlaid onto mouse X-Ray images, at 10° increments, using a 360° rotational imaging program, provided by Bruker BioSpin Molecular Imaging software. Images were acquired 10 minutes post-luciferin injection (150 mg/kg, intraperitoneal, bolus in 100 µL 1xPBS, luciferin from Targeting Systems). In-Vivo Xtreme II camera settings: for luminescent images: 10 sec, 2x2 bin, 1.1 f-stop, 12 cm x 12 cm FOV, and for X-Ray images: 10 sec, 2x2 bin, 2.8 f-stop, 12 cm x 12cm FOV.

## References

- [1] Gahan GC. (2012) The Bacterial Lux Reporter System: Applications in Bacterial Localization Studies. *Curr. Gene. Ther.* 12(1): 12-9.
- [2] Xu T, Close D, Handagama W, Marr E, Sayler G, Ripp S. (2016) The Expanding Toolbox of In Vivo Bioluminescent Imaging. *Front Oncol.* 6:150. doi: 10.3389/fonc.2016.00150.
- [3] Kim JE, Kalimuthu S, Ahn BC. (2015) In vivo cell tracking with bioluminescence imaging. *Nucl Med Mol Imaging.* 49(1):3-10. doi: 10.1007/s13139-014-0309-x.



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