#### **PRODUCT NOTE**

# revvity

### Create your own luciferase cell line with confidence.

### Key features

- Brighter red shifted firefly luciferase for better sensitivity *in vivo*
- Multiplex firefly luciferase with bright Green Renilla luciferase
- UbC promoter for stable luciferase expression
- Rapid, highly efficient and stable transduction
- Transduce mammalian cancer cells, stem cells, non-dividing cells and other cell types

IVISbrite<sup>™</sup> Lentiviral Particles are self-inactivating, recombination incompetent lentiviral particles carrying red-shifted firefly luciferase (*Luciola italica*) or green-shifted Renilla luciferase (*Renilla reniformis*) transgene under control of the stable UbC promoter. The firefly luciferase transgene is fused either to the puromycin resistance gene or Green Fluorescent Protein (GFP) gene via T2A "self-cleaving" linker peptide for efficient co-expression with selection marker. The green Renilla luciferase is also fused to the puromycin resistance gene via T2A "self- cleaving" linker peptide. The lentiviral particles are pseudotyped with G glycoprotein from Vesicular Stomatitis Virus (VSVG), allowing efficient transduction of a wide variety of mammalian cells including most cancer cell lines, primary, stem and non-dividing cells.

### Available IVISbrite Lentiviral Particles

- IVISbrite Red F-luc-Puromycin (Catalog # CLS960002)
- IVISbrite Red F-luc-GFP (Catalog # CLS960003)
- IVISbrite Green Renilla-Puromycin (Catalog # CLS960004)

Each vial contains at least 1 x  $10^7$  units/mL of lentiviral particles resuspended in 200  $\mu L$ 

IVISbrite lentiviral particles offer the most convenient way to create stably transfected cells to monitor tumor growth, track primary or stem cells *in vivo* and various other applications using IVIS *in vivo* imaging systems. The co-expression of GFP along with Red firefly luciferase makes it possible to monitor same cells *in vivo* using the luciferase reporter and ex vivo with GFP.

## Red shifted firefly luciferase Green Renilla luciferase

The firefly luciferase in the IVISbrite lentiviral particles originates from *Luciola italica*, which emits at 620 nm as compared to luciferase from *Photinus pyralis* that emits at 600nm (Figure 1A). The red emission may lead to better sensitivity of detection *in vivo*. The bioluminescent signal from cell lines transduced with red shifted luciferase is extremely bright allowing for better sensitivity when imaging at depth. The Green Renilla luciferase emits at 527 nm as compared to native Renilla luciferase that emits at 480 nm (Figure 1B). The green renilla luciferase is 35-40 fold brighter than native luciferase making it more attractive for imaging applications.



Figure 1: A) Comparison of the emission spectrums of Luc2 (Photinus pyralis) and Red F-luc (Luciola italica) luciferases. B) Emission spectrum of Green Renilla luciferase

### Stability of bioluminescent signal in vitro

The IVISbrite lentiviral particles incorporate ubiquitin (UbC) promoter for extremely stable reporter expression. Figure 2 shows the stability of firefly luciferase expression in 4T1 cells over a period of six weeks. The luciferase expression was very stable for almost six weeks. Other mammalian cancer cells like PC-3M (Human Prostate Cancer), Hep-G2 (Human Liver Cancer) and MDA-MB-231 (Human Breast Cancer) transduced with RediFect lentiviral particles also showed excellent stability over six week period.



Figure 2: 4T1 cells were infected with Red F-luc-Puro lentivirus and stably transduced cells were selected with puromycin. The intensity of the bioluminescent signal was determined with IVIS system once a week for 6 weeks.

#### Longitudinally monitor cell growth in vivo

The IVISbrite lentiviral particles allow for rapid, efficient and stable transduction of a wide variety of tumor cell lines. The superior brightness of the red shifted firefly luciferase is ideal for non-invasive tumor growth monitoring and early detection of micro metastases. Figure 3 shows longitudinal monitoring of PC-3M cells transduced with RediFect firefly luciferase lentiviral particles. High photon expression in cells transduced with RediFect firefly luciferase makes it easier to detect signal at depth in organs like lung and liver.



Figure 3: A)One million of PC3M Red-FLuc cells were injected s.c. into the right flank of a nude mouse. Tumors were imaged every two weeks with IVIS Spectrum. B) One million of A549 RedLuc lung tumor cells were injected into the lung of nude mouse. Eight weeks later mice were imaged with IVIS SpectrumCT and the tumor mass was reconstructed using Living Image® DLIT software.

### Green Renilla luciferase & dual reporter lentiviral particles

IVISbrite lentiviral particles offer the flexibility to transduce cells with widely used bioluminescent reporter like Renilla luciferase. The Green Renilla luciferase is optimized to emit in the 527 nm range as compared to 480 nm with native Renilla luciferase. With the wavelength shift and increased photon expression two reporters can be simultaneously monitored *in vivo* (Red Firefly luciferase and Green Renilla luciferase). Dual reporter cell lines can also be created with lentiviral particles containing firefly luciferase and GFP. Firefly luciferase is an excellent reporter for *in vivo* studies while GFP can be used for *in vitro* and *ex vivo* analysis. The GFP reporter in Red F-luc-GFP construct can be detected by FACS.



Figure 4: A) MDA-MB-231 cells were infected with Green-RenLuc-Puro lentivirus and stably transduced cells were selected using puromycin. The intensity of the bioluminescent signal was determined with IVIS system once a week for six weeks. B) Dual labeled MDA-MB-231 cells labeled with Red-Fluc and GFP. The GFP reporter expression can be detected by both FACS analysis. C) Measuring GFP expression in HT1080 cells using Nuance Multispectral Imaging System.

#### Disclaimer

IVISbrite Lentiviral particles are manufactured for Revvity by Targeting Systems, Inc. For research use only. Not for use in diagnostic procedures.

For more information, please visit our website at www.revvity.com





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