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LentiGlo[™] Prepackaged Lentivirus and Lentiplasmid Vectors (<u>Click here to view LentiGlo Presentation</u>) Custom lentivirus production and bioluminescent cell line service (<u>Click here to Enquire</u>)

Product Offering:

- Premade lentivirus and lentiplasmid vectors expressing Gaussia luciferase, the brightest, smallest known luciferase along with a fluorescent protein.
- · Lentivirus vectors expressing a very bright red-emitting Firefly luciferase from Luciola Italica
- Lentiviral vectors expressing Intracellular Green-emitting Renilla luciferase (35X brighter than native Renilla luciferase with an emission max at 527 nm)
- Lentiviral vectors with co-expression of luciferases with fluorescent proteins (choice of EGFP, mVermilionn or mScarlet. mScarlet and mVermilion (both monomeric) are very bright red fluorescent proteins

Advantages of Gaussia Luciferase and Red-emitting firefly and Green-emitting Renilla luciferase reporters:

- A bright red-emitting mutant of the Italian firefly Luciola Italica with an emission max of 617 nm offers advantage of a bright more stable bioluminescent signal and is great for imaging deep-seated tissues. Over 50 cancer cells lines have been stably transduced and tested in vivo
- Gaussia luciferase is the smallest, brightest known luciferase, 1000 times brighter than firefly or Renilla luciferase. Increased sensitivity for in vivo imaging applications, uses coelenterazine as substrate.
- Gaussia luciferase is actively secreted by mammalian cells so it is possible to quantitate growth of implanted ells by assaying luciferase activity in blood
- All our ready-to use lentiviruses are SIN (self-inactivating) vectors so once the virus integrates into the genome of the transduced cells it gets inactivated so it can no longer replicate.

Catalog No.	Name of LentiGlo lentivirus	Description	Price
LP-30	Lenti-UBC-RedFluc- t2A-Puro	Lentivirus expressing a very bright red-emitting luciferase (2X200 ul 10 ⁷ lentiviral particles per ml), pLenti-UBC-RedFluc-T2A-Puro lentiplasmid, 1000 assays of the firefly luciferase assay reagent	\$2000
LP-31	Lenti-UBC-RedFluc- t2A-EGFP	Lentivirus expressing Red firefly luciferase and GFP(2X200 ul 10 ⁷ lentiviral particles per ml), pLenti-CMV-RedFluc-IRES-EGFP plasmid, 1000 assays of the firefly luciferase assay reagent.	\$2000
LP-39	Lenti-UBC-GLuc-T2A- Puro	Lentivirus expressing secreted Gaussia luciferase (stable mutant) under control of the UBC promoter and puro resistance, pLenti-UBC-GLuc-T2A-Puro lentiplasmid, 1000 assays of Gaussia luciferase assay reagent GAR-1	\$2000
LP-42	Lenti-CMV-GLuc-T2A- RedFluc-T2A-Puro	Lentivirus expressing secreted Gaussia luciferase and intracellular Red firefly luciferase (2X200 ul 10 ⁷ lentiviral particles per ml),, pLenti-CMV-GLuc-T2A-RedFluc lentiplasmid, 1000 assays of the firefly luciferase assay reagent FLAR-1	\$2000
LP-43	Lenti-CMV-GLuc-T2A- EGFP	Lentivirus expressing secreted Gaussia luciferase along with EGFP under control of the CMV promoter (2X200 ul 10 ⁷ lentiviral particles per ml),, pLenti-CMV-GLuc-T2A-EGFP plasmid, 500 assays each of the Gaussia luciferase and firefly luciferase assay reagent FLAR-1	\$2000
LP-60	pLenti-UBC-GrRenLuc- T2a-Puro	Lentivirus expressing a very bright (30X brighter) Green Renilla luciferase under control of the UBC promoter (2X200 ul 10 ⁷ lentiviral particles per ml),, pLenti-UBC-GrRenLuc-T2A-Puro Lentiplasmid, 1000 assays of the Renilla luciferase assay reagent RLAR-1	\$2000
LP-62	pLenti-UBC-RedFluc- T2A-mVermillion	Lentivirus expressing Red firefly luciferase and mVermillion, a monomeric red fluorescent protein 2.5X brighter than mCherry pLenti-CMV-RedFluc-IRES-mVermillion plasmid, 1000 assays of the firefly luciferase assay reagent FLAR-1	\$2000
Lenti- Custom	Lenti-Custom	Any of the above expressing luciferase under your promoter of interest (subject to availability)	\$2500



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Figure 1: Human medulloblastoma cells transduced with a lentivirus co-expressing a red-emitting firefly luciferase along with RFP





Figure 2: Intrahepatic luciferase luminescence detected by real-time imaging (Xenogen IVIS® Spectrum Bioluminescence). **(1)** Strong luminescence signals are noted on the right upper abdomen of a mouse treated with magnet 14 days post intrahepatic injection of mEP cells labeled with UBC-RedFluc-tdtomato. **(2)** No signal seen in negative control. **Data courtesy of Dr Jeffrey Fair's lab, Cedar Sinai Hospital, Los Angeles, CA.**

Gaussia luciferase levels in blood are linear with respect to implanted cell number







Figure 3 : (Left) Different numbers of Gli36 human glioma cells expressing-Gluc (Gli36-Gluc) were implanted subcutaneously in mice and 3 days later, mice were injected i.v. with coelenterazine (4 mg/kg body weight) and imaged with CCD camera. (right) Total relative light units (RLU) per second was calculated for tumors in (red line). Gluc activity was measured in 5 µl blood (blue line) or urine (green) after addition of 100 µL 100 µM coelenterazine This figure appears in a publication -Wurdinger T, Pike L, Badr C, de Klein R, Weissleder R, Breakefield XO, Tannous BA. A secreted luciferase for ex vivo monitoring of in vivo processes Nature Methods 2008;5:171-173 (Journal cover) ADDITIONAL RESOURCES: POWERPOINT PRESENTSTION UNDER TECHNICAL RESOURCES SECTION ON OUR WEBSITE



Promoter Options:CMV and UBC Selection- Puromycin resistance or GFP Reporter Combinations : Red-emitting firefly luciferase, Gaussia luciferase and Green-emittign renilla luci LentiiGlo kits with the following lentiplasmids and packaged ready-to-use lentiviruses are available Red firefly luciferase-IRES-EGFP (catalog #LP-08) Red-firefly luciferase-T2A-Puro (catalog # LP-30) Red firefly luciferase-T2A-EGFP (catalog #LP31) Green Renilla luciferase-T2A-Puro (catalog # LP33) Gaussia luciferase-T2A-EGFP (catalog #LP-07N) Gaussia luciferase-T2A-Puro (catalog # LP-32)

All kits include ready to use lentivirus, the lentiplasmid and luciferase assay reagent (1000 assays)

- Alternate Luciferase reporter options:
- Bright red-emitting Luciola luciferase (emission max 617 nm)
- Green Renilla luciferase (40X brighter than commercially available blue-emitting Renilla luciferase, engineered for improved stability in vivo)
- Cypridina luciferase (secreted reporter)

For more information, click on teh links below or visit our website <u>http://www.targetingsystems.net</u> Related resources::

Poster presentation:

Applications for Gaussia luciferase for imaging of stem cells using LentiGlo. View poster presentation link on our website under "Technical Resources"

http://www.targetingsystems.net/technical-resources.html

Technology spotlight emphasizing use of Dr Branchini's red-emitting luciferase for tumor imaging. The redemitting

Luciola luciferase offered by us has been originally licensed from Dr Branchini's lab (Connecticut College) but is an improved version being about a 1000-fold brighter than the native Luciola luciferase http://www.rsc.org/Publishing/Journals/cb/Volume/2009/1/Light_up.asp

To place orders or for more information, please call us at 1(888)818-2446 or (619)562 1518 or FAX us at (619)562 1326. You can also contact us via email info@targetingsystems.net

Targeting Systems

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Licensing:: LentiGlo products and lentiplasmid vectors are sold for research purposes only and covered by multiple patents. For information regarding licensing issues please contact technology transfer by email info@targetingsystems.net 1-866-620-4018.

The red-emitting firefly luciferase from the Italian firefly Luciola Italica is covered by issued and pending patents held-licensed by Targeting Systems. Please contact Dr Rampyari Walia at 619 249-2457 or 619 562-1518 regarding licensing



Please store the product frozen at -80 ^oC

Product Protocol:

Cells are normally transduced cells at MOI (multiplicity of infection) of 10-50 depending on cell types. most humantumor cells infect really well at MOI10. polybrene does increase transduction efficiency, but some cells such as primary cells do not like it. you can obtain it from Sigma Chemicals, MO, USA.. We recommend using it at a 10ug/ml concentration (Polybrene is made up in PBS)

-Plate cells in 6-well plate at 70-80% confluency on day 1

-On day 2, remove media and add 1 ml of media containing lentivirus (MOI 10-50 use 20ul, 100 ul of Lentiprep provided) and 10 ug/ml polybrene (optional and not recommended for primary cells)

-On day 3 remove virus, wash cells and re-place with fresh media transfer cells to bigger plate once they are confluent.

-Assay for luciferase acitivty in the supernatant or examine cells for expression of fluorescent protein. Alternately intracellular luciferase acitivyt can be quantitated by lysing cells with the Targeting Systems Cell lysis buffer (catalog 5X CLR-01)

-Use luciferase assay protocol provided with the luciferase assay reagent that is supple dwith the LentiGlo kit. Please call tech support 1-866-620-4018 if you need more information or need clarification on the protocol