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Important Limited Use License information for pSELECT-zeo-Lucia

The purchase of the pSELECT-zeo-Lucia vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.



pSELECT-zeo-Lucia

A secreted luciferase reporter gene system selectable with Zeocin®

Catalog code: psetz-lucia https://www.invivogen.com/lucia-gene

For research use only

Version 23A19-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pSELECT-zeo-Lucia provided as lyophilized DNA
- 1 ml of Zeocin® (100 mg/ml)
- 1 tube of QUANTI-Luc™ 4 Reagent, a Lucia luciferase detection reagent (sufficient to prepare 25 ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20 °C and is stable for up to 1 year.
- Store Zeocin® at 4°C or at -20°C. The expiry date is specified on the product label.
- Store QUANTI-Luc[™] 4 Reagent at -20°C. After preparation, the working solution is stable for 48 hours at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles. *Note:* This product is photosensitive and should be protected from light.

Quality Control:

- Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

InvivoGen provides the Lucia luciferase reporter gene in the pSELECT-zeo plasmid. The plasmid pSELECT-zeo-Lucia can be used in vivo and in vitro to transfect mammalian cells stably or transiently. Lucia luciferase gene expression is driven by the EF-1 α /HTLV composite promoter that combines the elongation factor 1 alpha core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus. The pSELECT-zeo-Lucia plasmid contains the Zeocin® resistance marker for selection in both mammalian cells and bacteria.

Lucia luciferase is a completely novel and optimized luciferase with strong bioluminescent activity. It is expressed by a synthetic gene designed on natural secreted luciferase genes from marine copepods. Lucia luciferase is a secreted coelenterazine-utilizing luciferase that generates 1000-fold higher bioluminescent signal compared to the commonly used Firefly and *Renilla* luciferases. Lucia luciferase is designed for high and prolonged expression in mammalian cells.

Lucia luciferase activity can be determined using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagent.

PLASMID FEATURES

First expression cassette

- hEF1-HTLV promis a composite promoter comprising the Elongation Factor-1alpha (EF-1a) core promoter 1 and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat 2. The EF-1a promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1a core promoter to enhance stability of RNA.
- Lucia luciferase is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase. ORF size (from the ATG to the stop codon): 634 bp.

PLASMID FEATURES

- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- pMB1 Ori is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

Second expression cassette

- CMV enh/prom: The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters
- EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- *Sh ble*: Resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, Zeocin® can be used to select stable mammalian cells transfectants and *E. coli* transformants.
- **ßGlo pan:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.
- 1. Kim DW. et al., 1990. Use of the human elongation factor 1α promoter as a versatileand efficient expression system. Gene 91(2):217-23.2. Takebe Y. et al., 1988. SR alphapromoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. 3. Carswell S. & Alwine JC., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site:effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. 4. Yu J. & Russell JE.,2001. Structural and functional analysis of an mRNP complex that mediates the highstability of human β -globin mRNA. Mol Cell Biol. 21(17):5879-88.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 μ g/ μ l, resuspend the DNA in 20 μ l of sterile H_aO. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in commonly used laboratory E. coli strains, such as DH5 α .

Zeocin® usage

This antibiotic can be used for *E. coli* at 25-50 μ g/ml in liquid or solid media and at 50-200 μ g/ml to select Zeocin®-resistant mammalian cells.

QUANTI-Luc™ 4 Reagent

- Dilute the total volume of the 20X tube (1.25 ml) of QUANTI-Luc™ 4
 Reagent into 23.75 ml sterile water to obtain 25 ml of working solution.
 Vortex very briefly (a few seconds).
- 3. Use the working solution immediately or store until required for use.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Zeocin®	Selection antibiotic	ant-zn-1



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InvivoGen Asia: +852 3622-3480 E-mail: info@invivogen.com



QUANTI-Luc[™] 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23A16-MM

PRODUCT INFORMATION

plates (25 ml standard Flash/end-point detection).

Contents

1 tube of QUANTI-Luc[™] 4 Reagent (20X)
 One tube of QUANTI-Luc[™] 4 Reagent is sufficient for 5 x 96-well

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia kit.

QUANTI-Luc[™] 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc[™] 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc[™] 4 Stabilizer 25X (optimized Glow assay reagent)

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

- Store QUANTI-Luc[™] 4 Reagent at -20°C for up to 12 months.
- After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (pH, appearance).
- Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

MFTHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

- 1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
- 2. Vortex very briefly (a few seconds).
- 3. Use the working solution immediately or store until required for use. QUANTI-Luc[™] 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of luciferase activity from cell culture medium:

To obtain **end-point readings** using a luminometer **with an injector**.

- 1. Set the luminometer with the following parameters: 50 μl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
- 2. Pipet 20 μl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
- 3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.

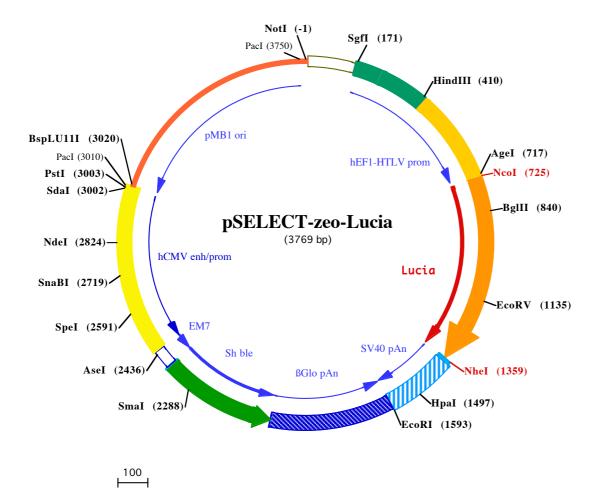
- 1. Set the luminometer with a 0.1 second reading time.
- 2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
- 3. Add 50 µl of QUANTI-Luc™ 4 Reagent 1X to each well or tube.
- 4. Gently tap the plate several times to mix (do **not** vortex).
- 5. Proceed immediately with the measurement.

RELATED PRODUCTS

Product	Cat. Code		
QUANTI-Luc™ 4 Lucia/Gaussia Kit			
500 tests	rep-qlc4lg1		
2 x 500 tests	rep-qlc4lg2		
5 x 500 tests	rep-qlc4lg5		



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NotI (-1)
  SgfI (171)
101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
201 GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATG
301 TCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGACCGTATATAAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGTTTGCCGCCAG
501
    NcoI (725)
                  AgeI (717)
CACCGGTCACCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAG
701 GGCGCCTACCTGAGATCAG
                         \textbf{1} \blacktriangleright \textbf{M} \ \textbf{E} \ \textbf{I} \ \textbf{K} \ \textbf{V} \ \textbf{L} \ \textbf{F} \ \textbf{A} \ \textbf{L} \ \textbf{I} \ \textbf{C} \ \textbf{I} \ \textbf{A} \ \textbf{V} \ \textbf{A} \ \textbf{E} \ \textbf{A} \ \textbf{K} \ \textbf{P} \ \textbf{T} \ \textbf{E} \ \textbf{I} \ \textbf{N} \ \textbf{E}
                                      BglII (840)
801 ACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACĀĀTCTTGAGACTGACCTGTTCACCAACTGGGAGACCATGAATGTGATTAGCACTGACAC
 25 D L N I A A V A S N F A T T D L E T D L F T N W E T M N V I S T D T
901 AGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCCTGAGGGAGCCGATGCCAGAAGGGCTGGT
     E O V N T D A D R G K L P G K K L P P D V L R E L E A N A R R A G
 58▶
1001 TGCACAAGAGGCTGCCTCATTTGCCTCCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGG
 92 CTRGCLICLSHIKCTPKMKKFIPGRCHTYEGEK
                                 EcoRV (1135)
1101 AGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGA
125▶E S A Q G G I G E A I V D I P E I P G F K D K E P L D Q F I A Q V D
1201 CCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTT
158 L C A D C T T G C L K G L A N V Q C S D L L K K W L P Q R C T T F
                                                      NheI (1359)
192 A S K I Q G R V D K I K G L A G D R •
                                                                                      HpaI (1497)
1401 ACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTATTTGTAACCATTATAAGCTGCAATAAACAAGTT
                                                                                  EcoRI (1593)
1601 AAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAAT
1701 GTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAA
1901 ATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAG
125◀ • D Q E E A V F H V C N G A P D R L A F E R G W P
                                                                         Q E G I
2101 CATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCCGCGTACAGCTCGTCCAGGCCGCGCACCCCACCCCAGGCCAGGGTG
            G S A D R F N T S V V E S W E A Y L E D L G R V W V W A L
                                                                              Smal (2288)
2201 TTGTCCGGCACCACCTGGTCCTGGACCGCGTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAAAACC
                                          V D D R V V G A F D D E
              V O D O V A
                                                                             D
27 L R D T W F E V A G A V D R A T
                                              L
                                                      VASTLKAM
                                   AseI (2436)
2401 AGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCT
                                                                                 SpeI (2591)
2501 GCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTTGGAAAGTCCCGTTGATTTACTAGTC
2600
                     SnaBI (2719)
2700 ATGGTAATAGCGATGACTAATACGTAĞATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCAT
                         NdeI (2824)
2800 TGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGT
              PacI (3010)
       PstI (3003)
      SdaI (3002)
                        BspLU11I (3020)
3000 ACGCCTGCAGGTT AA TTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGC
    {\sf CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG}
3198 TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCT
```

3298	${\tt CAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCCGACCGGTGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGGTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCACGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCACGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCACGACACACAC$
3398	AACCCGGTAAGACACGACTTATCGCCACTGGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGT
3498	GGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAAGAGTTGGTAGCTCTTGATCCGGCAA
3598	A CAAAC CACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGGAGAAAAAAAA
3698	PacI (3750) TCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAA

QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2 https://www.invivogen.com/quanti-luc

For research use only

Version 19A04-MM

PRODUCT INFORMATION

Contents

QUANTI-Luc™ is provided as packs of individually sealed pouches.

- rep-qlc1: 2 pouches of QUANTI-Luc™
- rep-qlc2: 5 pouches of QUANTI-Luc™

Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability

- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc $^{\sim}$ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

DESCRIPTION

QUANTI-Luc[™] is an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc[™] contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™

- 1. Pour the pouch contents into a 50 ml screw cap tube.
- 2. Add 25 ml of sterile water.
- 3. Swirl product gently until powder is completely dissolved.
- 4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

Detection of luciferase activity from cell culture medium

To obtain **end-point readings** using a luminometer **with an injector**.

- 1. Set the luminometer with the following parameters: 50 μ l of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
- 2. Pipet 10-20 μl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
- 3. Prime the injector with the QUANTI-Luc $^{\text{\tiny M}}$ assay solution and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.

- 1. Set the luminometer with a 0.1 second reading time.
- 2. Pipet 10-20 μ l of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
- 3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
- 4. Gently tap the plate several times to mix (do **not** vortex).
- 5. Proceed **immediately** with the measurement.

RFI ATFD PRODUCTS

Product	Catalog Code
QUANTI-Luc Gold (For standard and HTS assays) pSelect-zeo-Lucia (expression plasmid) Recombinant Lucia luciferase protein	rep-qlcg1 psetz-lucia rec-lucia
Reporter Cells THP1-Dual™ (IRF-Lucia/NF-ĸB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-кB Cells	thp1-nfkb

For a complete list of InvivoGen's Lucia luciferase Reporter Cell Lines visit https://www.invivogen.com/lucia-reporter-cells.

