

Before using this product, please read the Limited Use License statement below:

# 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 24H01-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
- 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-1α/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<sup>®</sup> 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 prom is a composite promoter comprising the Elongation Factor-1alpha (EF-1a) core promoter<sup>1</sup> 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<sup>2</sup>. 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  $1\alpha$  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 J.C., 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 µg/µl, resuspend the DNA in 20 µl of sterile H<sub>2</sub>O. 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 DH5α.

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

# QUANTI-Luc™ 4 Reagent

- 1. 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.
- 2. Vortex very briefly (a few seconds).
- 3. Use the working solution immediately or store until required for use.

Note: Lucia® is a registered trademark of InvivoGen.

# 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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# **QUANTI-Luc**<sup>™</sup> 4 Reagent

A coelenterazine-based luminescence assay reagent

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

For research use only

Version 24G30-MM

# PRODUCT INFORMATION

#### Contents

• 1 tube of QUANTI-Luc<sup>™</sup> 4 Reagent (20X) One tube of QUANTI-Luc<sup>™</sup> 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

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

Find more information at <a href="https://www.invivogen.com/quanti-luc">https://www.invivogen.com/quanti-luc</a>.

#### 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  $4^{\circ}\text{C}$  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  $^{\textcircled{\$}}$  protein or reporter cells.

#### DESCRIPTION

QUANTI-Luc<sup>™</sup> 4 Reagent is one component of the QUANTI-Luc<sup>™</sup> 4 Lucia/Gaussia and QUANTI-Luc<sup>™</sup> 4 Renilla kits. It contains the coelenterazine substrate for the detection of secreted Lucia<sup>®</sup> 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).

Note: Lucia  $^{\mathbb{R}}$  is a registered trademark of InvivoGen.

#### **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<sup>™</sup> 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

# Flash detection of Lucia<sup>®</sup> luciferase activity in cell culture medium:

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

- 1. Set the luminometer with the following parameters: 50  $\mu l$  of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
- 2. Pipet  $10\text{-}20\,\mu\text{l}$  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.

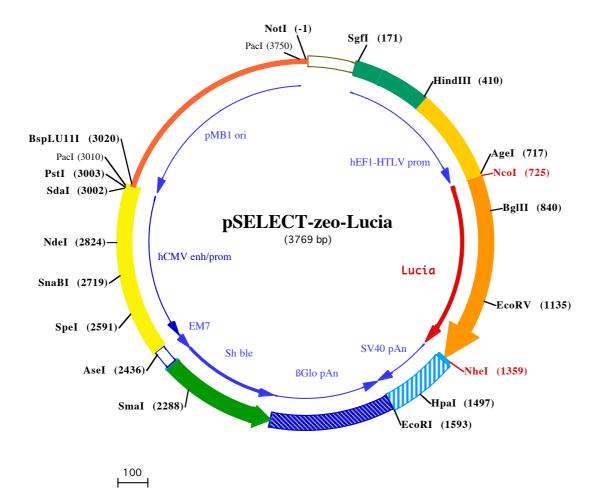
To obtain **end-point readings** using a luminometer **without injectors**.

- 1. Set the luminometer with a 0.1 second reading time.
- 2. Pipet 10-20 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 comprising QUANTI-Luc™ 4 Reagent & Stabilizer	rep-qlc4lg1
QUANTI-Luc <sup>™</sup> 4 Renilla Kit comprising QUANTI-Luc <sup>™</sup> 4 Reagent & Lysis buffer	rep-qlc4r1





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