pGLuc Mini-TK 2 Vector

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N8086S 006150518051

N8086S

20 µg **Lot: 0061505** **Exp: 5/18**

0.5 µg/µl **Store at –20°C**

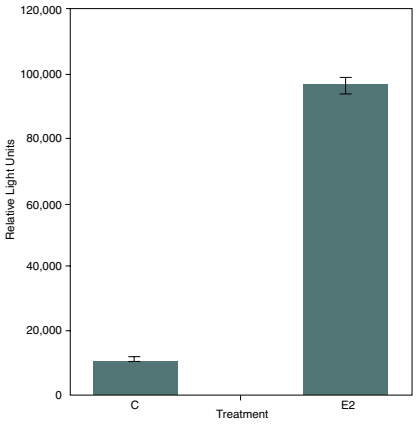
Description: pGLuc Mini-TK 2 is a cloning vector for mammalian cells, containing a minimal promter fragment from the HSV thymidine kinase (TK) promoter adjacent to a reporter gene, the secreted luciferase from the copepod *Gaussia princeps*. *Gaussia* Luciferase (GLuc) is a 19 kDa protein encoded by a "humanized" sequence, and it contains a native signal peptide at the N-terminus that allows it to be secreted from mammalian cells into the cell culture medium (1,2). The pGLuc Mini-TK 2 Vector contains a multiple cloning site (MCS) upstream of the minimal TK promoter for cloning promoter or enhancer elements. A neomycin resistance gene under the control of an SV40 promoter allows selection for stable integration of the plasmid into the mammalian cell genome using G418.

Source: Isolated from *E. coli* strain NEB10β by a standard DNA purification procedure.

Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM EDTA.

Advantages:

- Multiple samples can be obtained from the same transfected cells (i.e., before and after experimental treatments or at multiple time points).
- 90–95% of GLuc activity is found in the cell culture medium, with the remaining 5–10% detectable in cell lysates. This allows flexibility when assaying GLuc along with other co-transfected reporters.
- The activity of GLuc is high and the GLuc assay is sensitive enough to detect very small amounts of GLuc enzyme activity.
- GLuc is very stable in the cell culture medium so the GLuc activity detected reflects the amount of GLuc secreted by the transfected cells over a period of several days. GLuc can also be stored at 4°C for several days without any loss in activity.

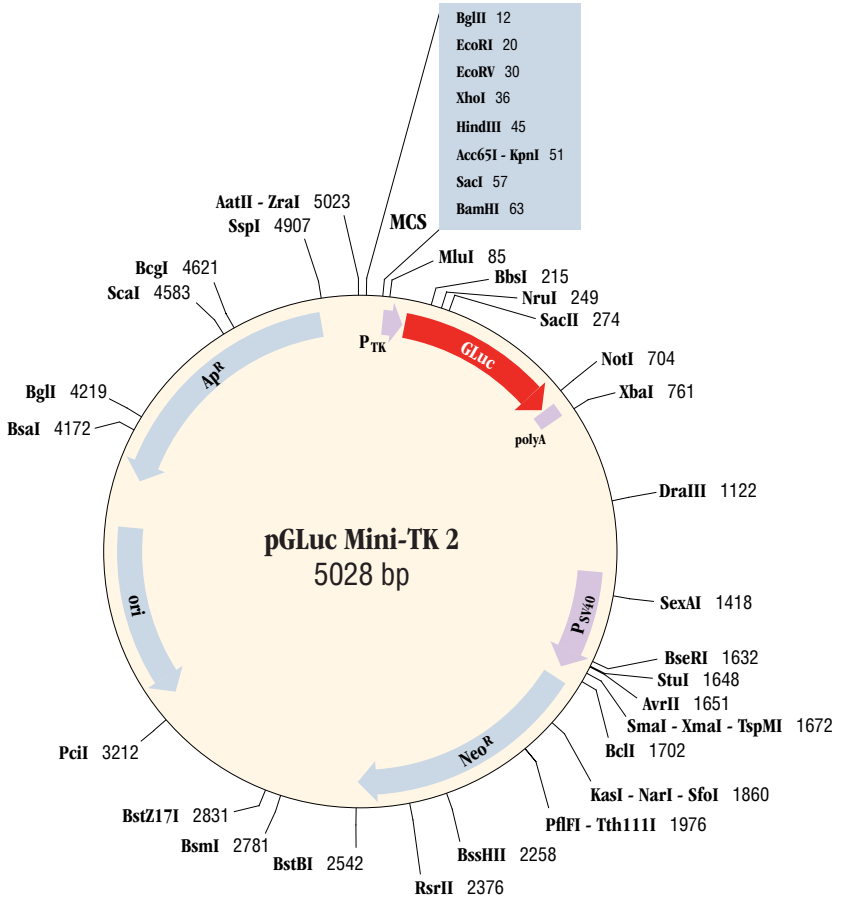


A DNA genomic fragment containing putative estrogen response elements was inserted into the polylinker of pGLuc Mini-TK 2 Vector. The properties of this fragment were assessed in a transfection assay. GLuc activity was measured in the culture supernatant of MCF-7 cells transfected with the construct and treated with vehicle control (C) or estradiol (E2).

- GLuc does not use the same substrate as *Cypridina* Luciferase. Therefore, it is possible to assay both GLuc and CLuc independently in cell culture medium from cells expressing both reporters (3,4).
- The pGLuc Mini-TK 2 Vector can be transfected into cells using any standard transfection protocol and stable cell lines can be established using Neomycin selection.

Applications:

- The pGLuc Mini-TK 2 Vector can be used to test promoter or enhancer elements by cloning into the MCS upstream of the minimal TK promoter. For constitutive expression of GLuc, vectors containing promoters are available (see Companion Products Sold Separately).
- GLuc can be used as a stand alone reporter or in conjunction with other compatible reporters such as *Cypridina* Luciferase (CLuc) (3). GLuc and CLuc are ideally suited for co-expression as both are secreted and highly active enzymes providing ease of use and sensitivity (3,4).



Restriction map of pGLuc Mini-TK Vector. Only unique restriction sites are shown. The complete sequence and restriction map is available at: http://www.neb.com/nebecomm/tech reference/

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      BglII    EcoRI      EcoRV  XhoI    HindIII Acc65I SacI
11    GAGATCTTGG AATTCTGCAG ATATCCTCGA GCCCAAGCTT GGTACCGAGC   60

        BamHI
61    TCGGATCCTT CGCATATTAA GGTGACGCGT GTGGCCTCGA ACACCGAGCG   110

                                              M  G   V  K  V
111   ACCCTGCAGC GACCCGCTTA AAAGATCCAG CCACCATGGG AGTCAAAGTT   160

      L
161   CTG...
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In pGLuc Mini-TK Vector the multiple cloning site precedes the minimal TK promoter (purple). The TATA box is underlined and the first amino acids of GLuc are indicated in the above sequence.

(see other side)

CERTIFICATE OF ANALYSIS

Features of pGLuc Mini-TK 2 Vector:

- Polylinker MCS: 12–68
- Minimal promoter from Herpes simplex virus Thymidine Kinase (Mini-TK): 69–131
- GLuc coding: 146–703
- Start codon: 146–148
- Stop codon: 701–703
- Signal peptide: 146–196
- Synthetic poly-A site: 712–760
- Neo promoter (SV40): 1346–1681
- Neomycin resistance gene: 1733–2527
- Bacterial replication ori (pMB1): 3861–3273
- Amp resistance: 4892–4032
- All pGLuc-2 vectors have improved poly-adenylation-transcription termination of the luciferase transcript. The polyadenylation signal is a synthetic polyadenylation sequence based on the β-globin gene (5).

Recommended Sequencing Primers for pGLuc Mini-TK 2 Vector (not available from NEB)

Upstream of MCS (23-mer):
GGGGTTCCGCGCACATTTCCCCG (4987–5009)

pBasic Reverse Primer (25-mer)
TCAGAAGCCATAGAGCCCACCGCAT (855–831)

GLuc 3´ end Forward Primer (20-mer)
GCCAGCAAGATCCAGGGCCA (650–669)

GLuc 5´ End Reverse Primer (24-mer)
TCAGGGCAAACAGAACTTTGACTC (173–150)

Frequently Asked Questions:

Where can I find the sequence of this plasmid?

The sequences of all the plasmids sold by NEB are available online at: http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/dna_sequences_maps.asp.

Can I generate a stable cell line with pGLuc Mini-TK 2 Vector?

Yes. Selection for neomycin resistant colonies after transfection can be carried out by growing the cells in media containing G418.

Can I transfect this plasmid into mammalian cells?

Yes. In general, fro transfection one will need to use plasmid DNA from CsCl prep or Qiagen® Maxi Prep.

How do I assay for GLuc expression?

Both the BioLux® *Gaussia* Luciferase Assay Kit (NEB #E3300) and the BioLux *Gaussia* Luciferase Flex Assay Kit (NEB #E3308) can be used to detect GLuc expression.

Is there another secreted reporter that can be used with GLuc?

Yes. *Gaussia* and *Cypridina* are both secreted luciferases, which produce high bioluminescent signal intensity. They oxidize different substrates that do not cross-react with each other. Therefore, *Gaussia* and *Cypridina* are an ideal duo for co-transfecting mammalian cells (2,3). Refer to the BioLux *Cypridina* Luciferase (CLuc) Assay Kits and CLuc expression vectors for more information.

References:

1. Verhaegen, M and Christopoulos, T.K. (2002) *Anal. Chem.,* 74, 4378–4385.
2. Tannous, B.A. et al. (2005) *Mol. Ther.,* 11, 435–443.
3. Otsuji, et al. (2004) *Anal. Biochemistry,* 329, 230–237.
4. Wu, et al. (2007) *Biotechniques,* 42, 290–292.
5. Levitt, et al. (1989) *Genes Dev.,* 3, 1019–1025.

Companion Products Sold Separately:

BioLux *Gaussia* Luciferase Assay Kit
#E3300S 100 assays
#E3300L 1,000 assays

BioLux GLuc Flex Assay Kit
#E3308S 100 assays
#E3308L 1,000 assays

Luciferase Cell Lysis Buffer
#B3321S 25 ml

pGLuc-Basic 2 Vector
#N8082S 20 µg

pCMV-GLuc 2 Control Plasmid
#N8081S 20 µg

pSV40-GLuc Control Plasmid
#N0323S 20 µg

pTK-GLuc Vector
#N8084S 20 µg

BioLux *Cypridina* Luciferase Assay Kit
#E3309S 100 assays
#E3309L 1,000 assays

pCLuc-Basic 2 Vector
#N0317S 20 µg

pCLuc Mini-TK 2 Vector
#N0324S 20 µg

pCMV-CLuc 2 Control Plasmid
#N0321S 20 µg

pSV40-CLuc Control Plasmid
#N0318S 20 µg

pTK-CLuc Vector
#N0322S 20 µg