pGLuc Mini-TK 2 Vector

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 promoter 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.

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.

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).

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/
Can I transfet this plasmid into mammalian cells? Yes. In general, for 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.

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

Upstream of MCS (23-mer):
GGGGTTCCGCGCAATTCTCCCG (4987–5009)
pBasic Reverse Primer (25-mer)
TCAGAAGCATAGGCGCCAGGCAT (855–831)

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

GLuc 5’ End Reverse Primer (24-mer)
TCAGGGCAACAGAATTTGACTC (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/ttech_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.

References:

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

pGLuc-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