pGLuc-Basic 2 Vector

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N8082S 009160919091

N8082S

20 µg **Lot: 0091609** **Exp: 9/19**

0.5 µg/µl **Store at –20°C**

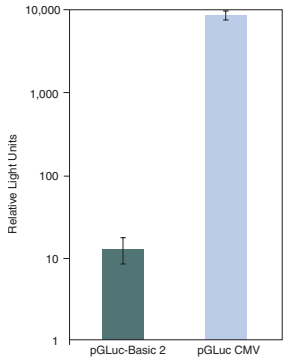
Description: pGLuc-Basic 2 is a cloning vector for expression in mammalian cells, containing a reporter gene but lacking promoter elements. The reporter gene is the secreted luciferase from the copepod *Gaussia princeps*. *Gaussia* Luciferase (GLuc) is a 19 kDa protein encoded by a "humanized" sequence, 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-Basic 2 Vector contains a multiple cloning site (MCS) upstream of the GLuc coding sequence. 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 an *E. coli* strain NEB10β by 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.

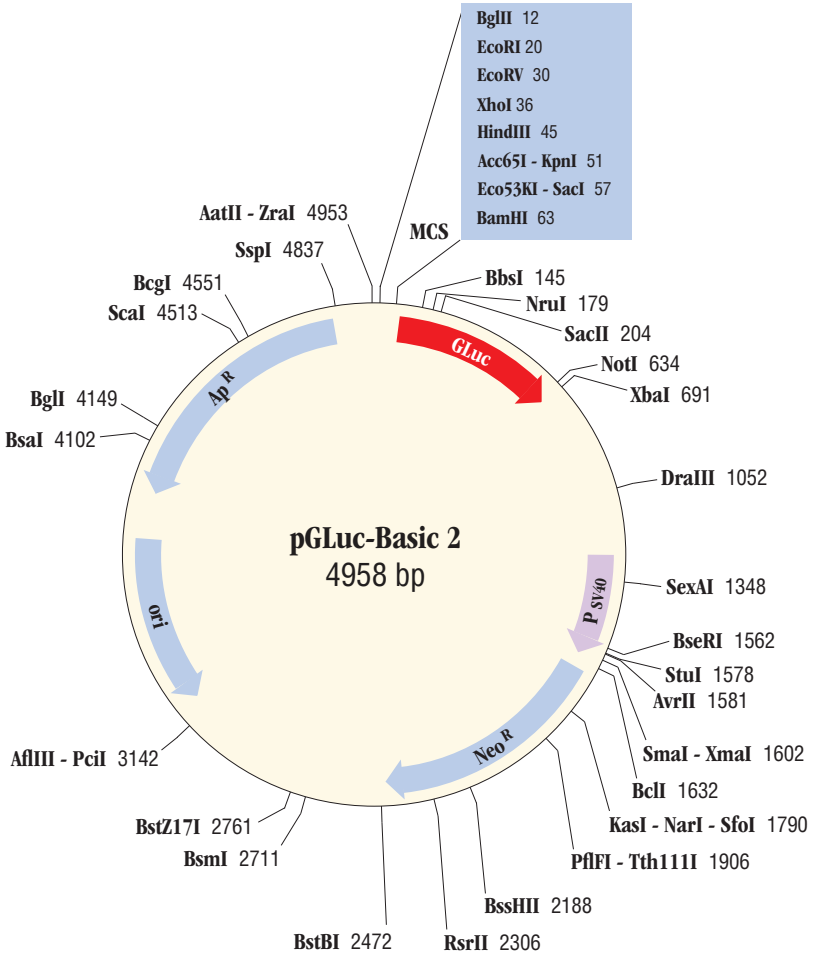


Comparison of light output obtained from HEK293 cells transfected with either the promoterless pGLuc-Basic 2 Vector or the pCMV-GLuc 2 Control Plasmid (NEB #N8081). Supernatants were harvested at 24 hour post-transfection and assayed for the GLuc activity using the BioLux GLuc Assay System.

- 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).
- The pGLuc-Basic 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-Basic 2 Vector can be used to test promoters by cloning promoter element of interest into the MCS upstream of the GLuc reporter gene. 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-Basic Vector and polylinker sequence. 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
 1   GACGGATCGGGAGATCTTGGAATTCTGCAGATATCCTCGAGCCCAAGCTT    50

     KpnI  SacI  BamHI
51   GGTACCGAGCTCGGATCCAGCCACCATGGGAGTCAAAGTTCTGTTTGCCC   100
                                 M  G  V  K  V  L  F  A...
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GLuc

pGLuc-Basic multiple cloning site (MCS). The Gaussia Luciferase sequence is shown with a blue background. Only unique restriction sites are shown.

(see other side)

Features of pGLuc-Basic 2 Vector:

- Polylinker MCS: 12–68
- GLuc coding: 76–633
- Start codon: 76–78
- Stop codon: 631–633
- Signal peptide: 76–126
- Synthetic poly-A site: 642–690
- Neo promoter (SV40): 1276–1611
- Neomycin resistance gene: 1663–2457
- Bacterial replication ori (pMB1): 3791–3203
- Amp resistance: 4822–3962
- All pGLuc 2 vectors and plasmids have improved polyadenylation-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-Basic 2 Vector (not available from NEB)

Upstream of MCS: (23-mer)
5´-GGGGTTCCGCGCACATTTCCCCG-3´(4917–4939)

pBasic Reverse Primer (25-mer)
5´-TCAGAAGCCATAGAGCCCACCGCAT-3´(785–761)

GLuc 3´ End Forward Primer (20-mer)
5´-GCCAGCAAGATCCAGGGCCA-3´(580–599)

GLuc 5´ End Reverse Primer (24-mer)
5´-TCAGGGCAAACAGAACTTTGACTC-3´(103–80)

Frequently Asked Questions:

Where can I find the sequence of this plasmid?

The sequences of all the vectors sold by NEB are available online at www.neb.com.

Can I generate a stable cell line with pGLuc-Basic 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, 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 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

Luciferase Cell Lysis Buffer
#B3321S 25 ml

pGLuc Mini-TK 2 Vector
#N8086S 20 µg

pCMV-GLuc 2 Control Plasmid
#N8081S 20 µg

pSV40-GLuc Control Plasmid
#N0323S 20 µg

pTK-GLuc Vector
#N8084S 20 µg

Anti-GLuc Antibody
#N8023S 0.2 ml

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

BioLux *Cypridina* Luciferase Starter Kit
#E3314S 100 assays
#E3314L 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