pCMV-GLuc 2 Control Plasmid

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N8081S 0111611119111

N8081S

20 µg **Lot: 0111611** **Exp: 11/19**

0.5 µg/µl **Store at –20°C**

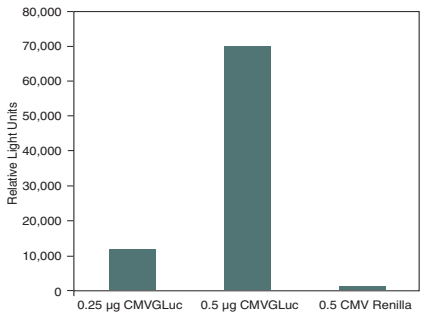
Description: The pCMV-GLuc 2 Control Plasmid is a mammalian expression vector that encodes the secreted luciferase from the copepod *Gaussia princeps* as a reporter, under the control of the constitutive CMV (cytomegalovirus) promoter. *Gaussia* luciferase (GLuc) is a 19 kDa protein encoded by a "humanized" 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). 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.



Comparison of light output obtained from secreted GLuc versus Renilla luciferase in U2OS cells that were transiently transfected with the corresponding expression vectors. Supernatants (GLuc) and lysates (Renilla) were harvested at 24 hour post-transfection and assayed for luciferase activity using the BioLux GLuc Assay System.

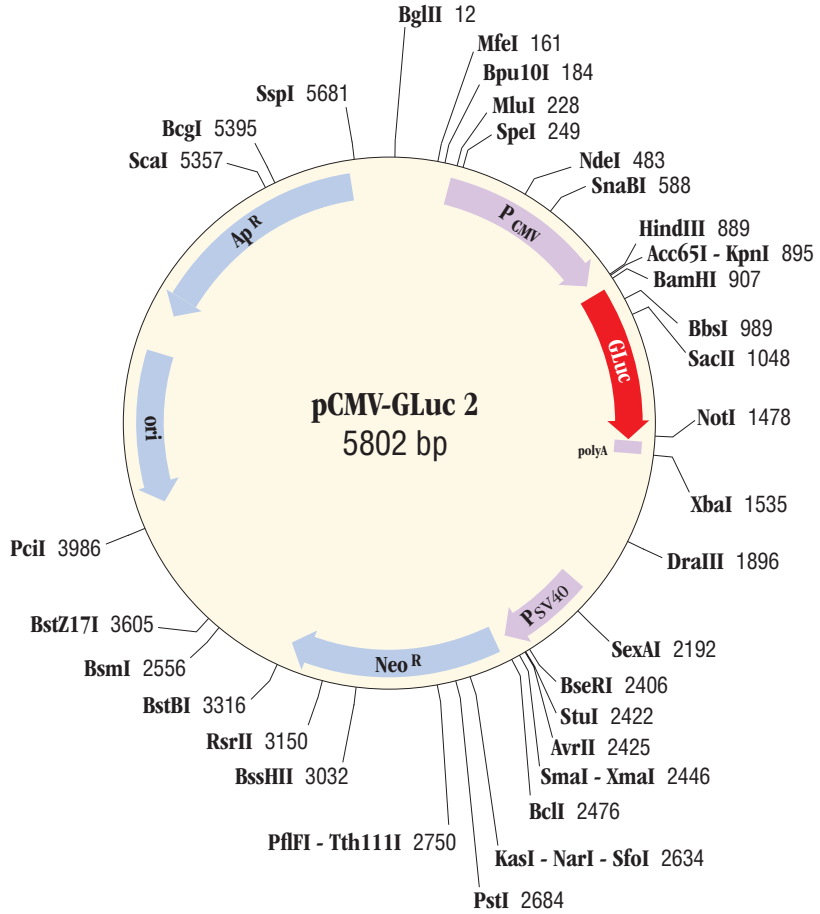
- GLuc does not use the same substrate as *Cypridina* luciferase. Therefore, it is possible to assay both GLuc and CLuc independently in cell culture medium from cells expressing both reporters (3,4).
- The pCMV-GLuc 2 Control Plasmid can be transfected into cells using any standard transfection and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

Applications:

- The pCMV-GLuc 2 Control Plasmid can be used as a control for assessing the efficiency of transfection in mammalian cells. Plasmids containing other constitutive promoter elements are also 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).

Features of pCMV-GLuc 2 Control Plasmid:

- CMV promoter: 209–863
- GLuc coding: 920–1477
- Start codon: 920–922
- Stop codon: 1475–1477
- Signal peptide: 920–970
- Synthetic poly-A site: 1486–1534
- Neo promoter (SV40): 2120–2455
- Neomycin resistance gene: 2507–3301
- Bacterial replication ori (pMB1): 4635–4047



Restriction map of pCMV-GLuc Vector. Only unique restriction sites are shown. The complete sequence and restriction map is available at: http://www.neb.com/nebecomm/tech_reference/

- Amp resistance: 5666–4806
- All pGLuc-2 vectors 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 pCMV-GLuc 2 Control Plasmid (not available from NEB)

T7 Universal Primer (20-mer)
TAATACGACTCACTATAGGG (863–882)

pBasic Reverse Primer (25-mer)
TCAGAAGCCATAGAGCCCACCGCAT (1629–1605)

GLuc 3´ End Forward Primer (20-mer)
GCCAGCAAGATCCAGGGCCA (1424–1443)

GLuc 5´ End Reverse Primer (24-mer)
TCAGGGCAAACAGAACTTTGACTC (947–924)

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: https://www.neb.com/tools-and-resources/interactive-tools/dna-sequences-and-maps-tool.

Can I generate a stable cell line with pCMV-GLuc 2 Control Plasmid?

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.

(see other side)

CERTIFICATE OF ANALYSIS

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-Basic 2 Vector
#N8082S 20 µg

pGLuc Mini-TK 2 Vector
#N8086S 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

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