pSV40-GLuc Control Plasmid

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N0323S 004160919091

N0323S

20 µg **Lot: 0041609** **Exp: 9/19**

0.5 µg/µl **Store at –20°C**

Description: The pSV40-GLuc 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 SV40 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). There is a multiple cloning site (MCS) upstream of the SV40 promoter.

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 (Figure 1). 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 (Figure 2).
- 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 pSV40-GLuc Control Plasmid can be transfected into cells using any standard transfection and stable cell lines can be established using Neomycin selection.

Applications:

- The pSV40-GLuc 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 pSV40-GLuc Control Plasmid:

- Polylinker MCS: 1–50
- SV40 promoter: 51-246
- GLuc coding: 306–863
- Start codon: 306–308
- Stop codon: 861–863
- Signal peptide: 306–356
- SV40 poly-A site: 890–1111
- SV40 enhancer: 1117–1363
- Bacterial replication ori (pMB1): 2269–1681
- Amp resistance: 3300–2440

Recommended Sequencing Primers for pSV40-GLuc Control Plasmid (not available from NEB)

GLuc 3´ End Forward Primer (20-mer)
GCCAGCAAGATCCAGGGCCA (810–829)

GLuc 5´ End Reverse Primer (24-mer)
TCAGGGCAAACAGAACTTTGACTC (333–310)

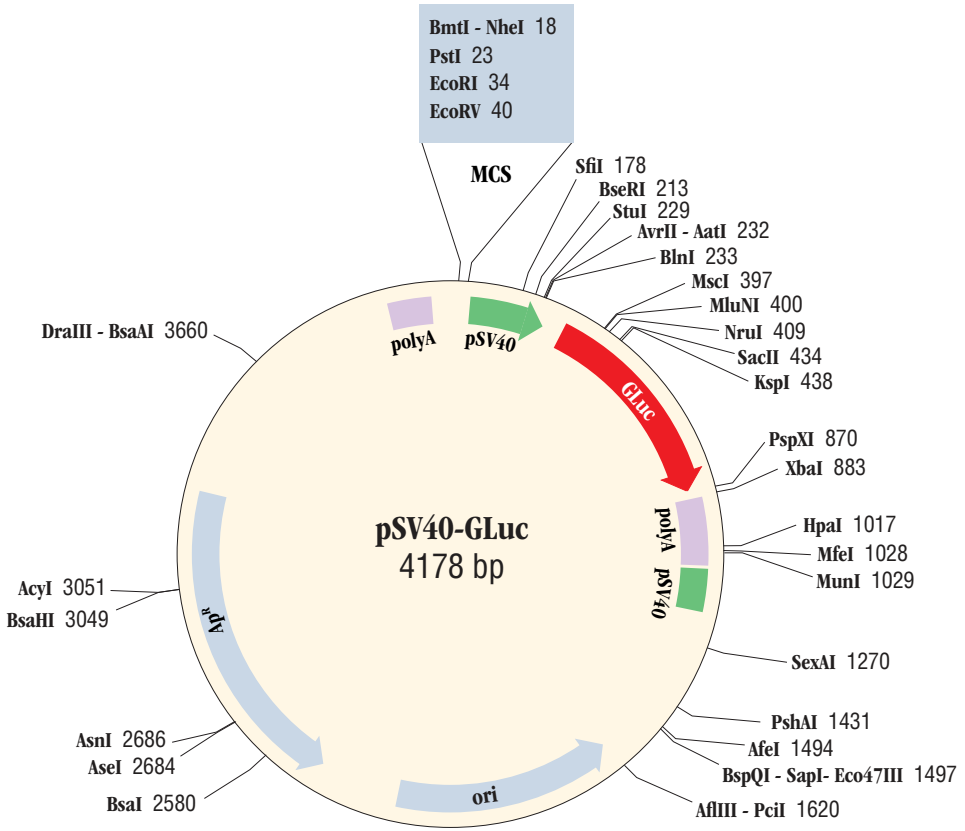
Frequently Asked Questions:

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 pSV40-GLuc Control Plasmid?

No. The pSV40-GLuc Control Plasmid does not contain a Neo^R marker for selection in mammalian cells.



Restriction map of pSV40-CLuc Control Plasmid 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/

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.

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CERTIFICATE OF ANALYSIS

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

pCMV-GLuc 2 Control Plasmid
#N8081S 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

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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