

pSV40-CLuc Control Plasmid



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N0318S 005140217021

N0318S

20 µg Lot: 0051402 Exp: 2/17

0.5 µg/µl Store at -20°C

Description: The pSV40-CLuc Control Plasmid is a mammalian expression vector that encodes the secreted luciferase from the Ostracod *Cypridina noctiluca* (1) as a reporter, under the control of the constitutive SV40 promoter. *Cypridina* Luciferase (CLuc) is a 62 kDa protein with a native signal peptide at the N-terminus that allows it to be secreted from mammalian cells (1) so that CLuc activity can be detected in the culture medium. There is a multiple cloning site (MCS) upstream of the SV40 promoter.

Source: Isolated from an *E. coli* strain NEB10β by standard DNA purification methods.

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 CLuc activity is found in the cell culture medium, with the remaining 5–10% detectable in cell lysates (Figure 1). This allows flexibility when assaying CLuc along with other co-transfected reporters.
- The activity of CLuc is high and the CLuc assay is sensitive enough to detect very small amounts of CLuc enzyme activity (Figure 2).
- CLuc does not use the same substrate as other marine luciferases (e.g. *Renilla*, *Gaussia*). Therefore, it is possible to assay both CLuc and GLuc independently in cell culture medium from cells expressing both reporters.
- The pSV40-CLuc Control Plasmid can be transfected into cells using any standard transfection protocol.

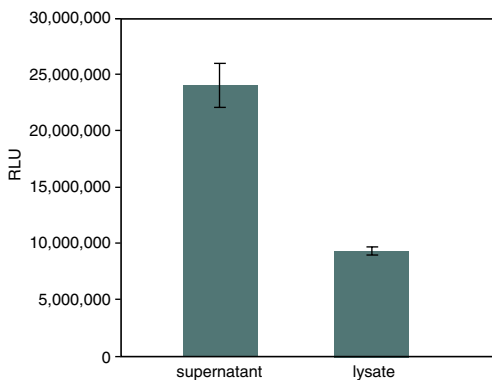


Figure 1: Activity of *Cypridina* Luciferase in supernatants and lysates from a stable CLuc-expressing cell line. CLuc activity was measured from 20 µl of cell culture supernatant (500 µl total culture volume) and from 20 µl of cell lysate (100 µl total lysate volume).

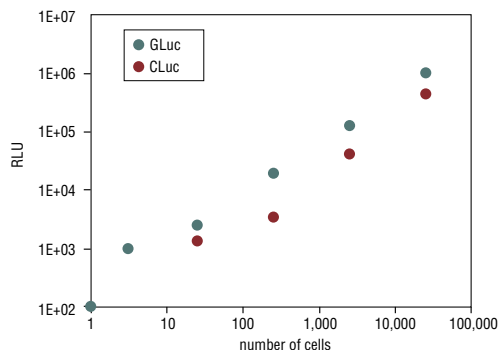
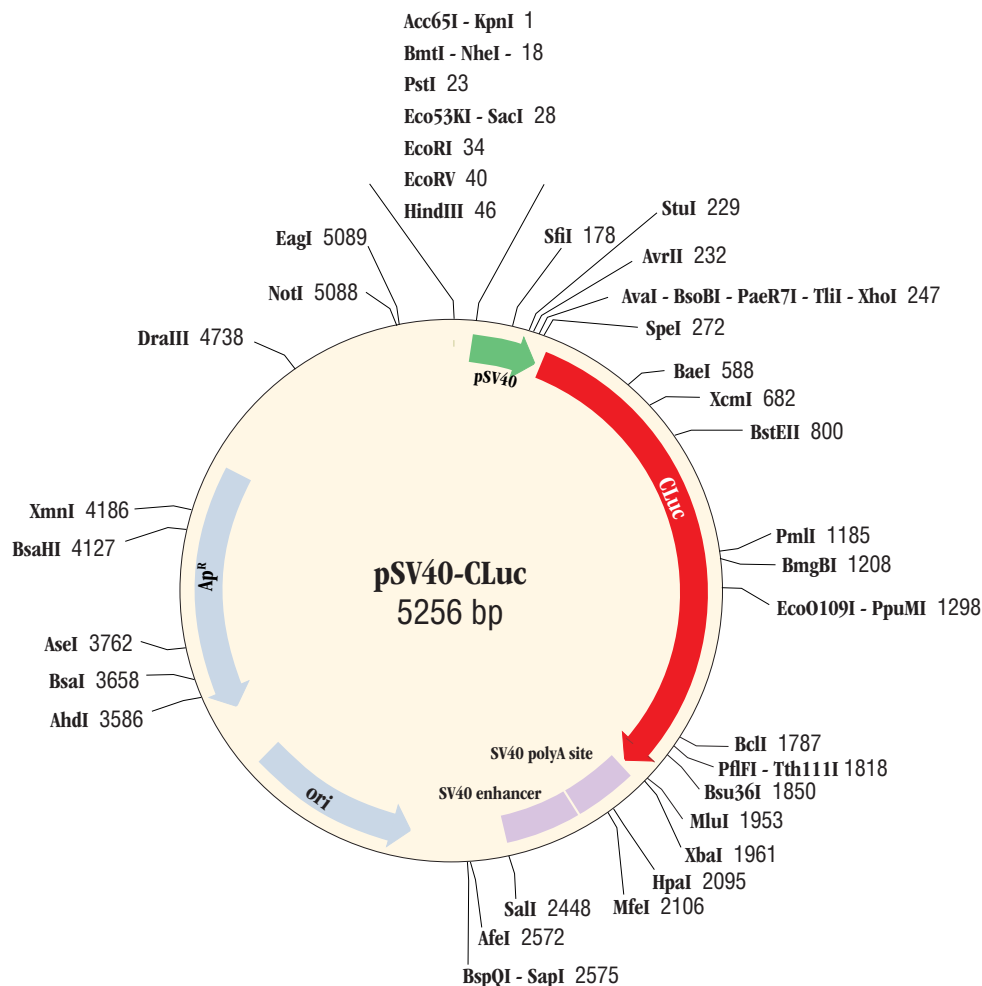


Figure 2: The high sensitivity of both the CLuc and GLuc assays allows detection of very small numbers of cells expressing each protein. 20 µl of culture supernatant from the indicated number of cells expressing each reporter were assayed.

Applications:

- The pSV40-CLuc 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). CLuc can be used as a stand alone reporter or in conjunction with other compatible reporters such as *Gaussia* luciferase (GLuc)(2). CLuc and GLuc are ideally suited for co-expression as both are secreted and highly active enzymes providing ease of use and sensitivity.



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/

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          PstI      EcoRI
KpnI          NheI   Eco53KI   HindIII
Acc65I        BmtI   SacI      EcoRV
1  GGTACCACCGATCGATCGCTAGCTGCAGAGCTCGAATTCGATATCAAGCTTGCATCTCAATTAGTCAGC  70
                                     SV40 promoter
    
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pSV40-CLuc multiple cloning site (MCS). The *Cypridina* Luciferase sequence is located downstream of the SV40 promoter (not shown); unique restriction sites are shown.

(see other side)

Features of pSV40-CLuc Control Plasmid:

- Polylinker MCS: 1–51
- SV40 promoter: 51–246
- CLuc ORF: 291–1952
- Start codon of CLuc: 291–293
Stop codon: 1950–1952
- Signal peptide: 291–344
- SV40 poly-A site: 1967–2188
- SV40 enhancer: 2195–2441
- Bacterial replication ori (pMB1): 3347–2759
- Amp resistance: 4378–3518

Recommended sequencing primers for pSV40-CLuc

CLuc:

Forward primer (23-mer) (not available from NEB)
5'-GAGTTCAAGAAAGAATGCTACAT-3' (1888–1910)

Reverse primer (24-mer) (not available from NEB)
5'-GTAAGGACAGTCCTGGCAATGAAC-3' (360–337)

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 www.neb.com.

Can I make a stable cell line with pSV40-CLuc?

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

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 CLuc expression?

Please refer to the BioLux[®] CLuc Assay Kit (NEB #E3309).

Can I use assay kits designed for other reporters (Gaussia, Renilla & Firefly luciferases) to assay CLuc activity?

No. *Cypridina* Luciferase catalyzes the light reaction using a different substrate that is not the same as those for *Gaussia*, *Renilla* & Firefly luciferases. Therefore, the CLuc activity can only be assayed by using the BioLux CLuc Assay Kit (NEB #E3309).

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

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

References:

1. Nakajima, Y. et al. (2004) *Biosci. Biotechnol. Biochem.* 63, 565–570.
2. Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y. (2007) *BioTechniques.* 42, 290–292.

Companion Products Sold Separately:

BioLux *Cypridina* Luciferase Assay Kit

#E3309S	100 assays
#E3309L	1,000 assays

pCLuc-Basic 2 Vector	
#N0317S	20 µg

Luciferase Cell Lysis Buffer	
B3321S	25 ml

BioLux <i>Gaussia</i> Luciferase Assay Kit	
#E3300S	100 assays
#E3300L	1,000 assays

BioLux <i>Gaussia</i> Luciferase Flex Assay Kit	
#E3308S	100 assays
#E3308L	1,000 assays

pCMV-GLuc 2 Control Plasmid	
#N8081S	20 µg

pGLuc Basic 2 Vector	
#N8082S	20 µg

pTK-GLuc Vector	
#N8084S	20 µg

pGLuc Mini-TK 2 Vector	
#N8086S	20 µg



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U.S. Patent Nos. 7,718,389; 7,989,621; 8,367,357 and 8,343,729
Japanese Patent Nos. 4,761,150 and 4,484,429
Japanese Appln. Serial No.: 2006-280827; 2007-536587 and 2009-257631
EPO Appln. Serial No.: 06 810 525.3
Chinese Appln. Serial No.: 200680035410.3

For use of the Biolum *Cypridina* Luciferase Assay Kit, or associated assay reagents, in human diagnosis and measurement in relation to human health, contact busdev@neb.com.