

pSV40-CLuc Control Plasmid



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N0318S

20 µg Lot: 0061505 Exp: 5/18
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.
- 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.

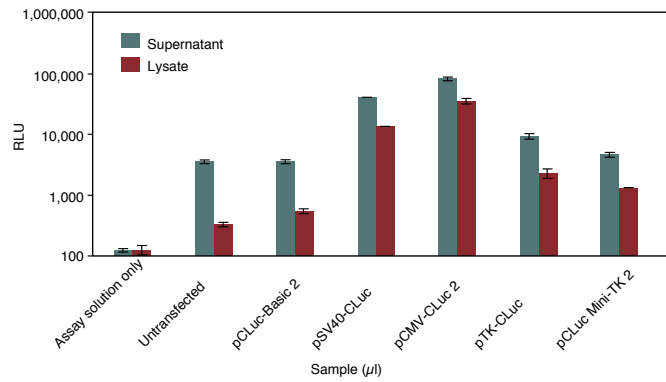


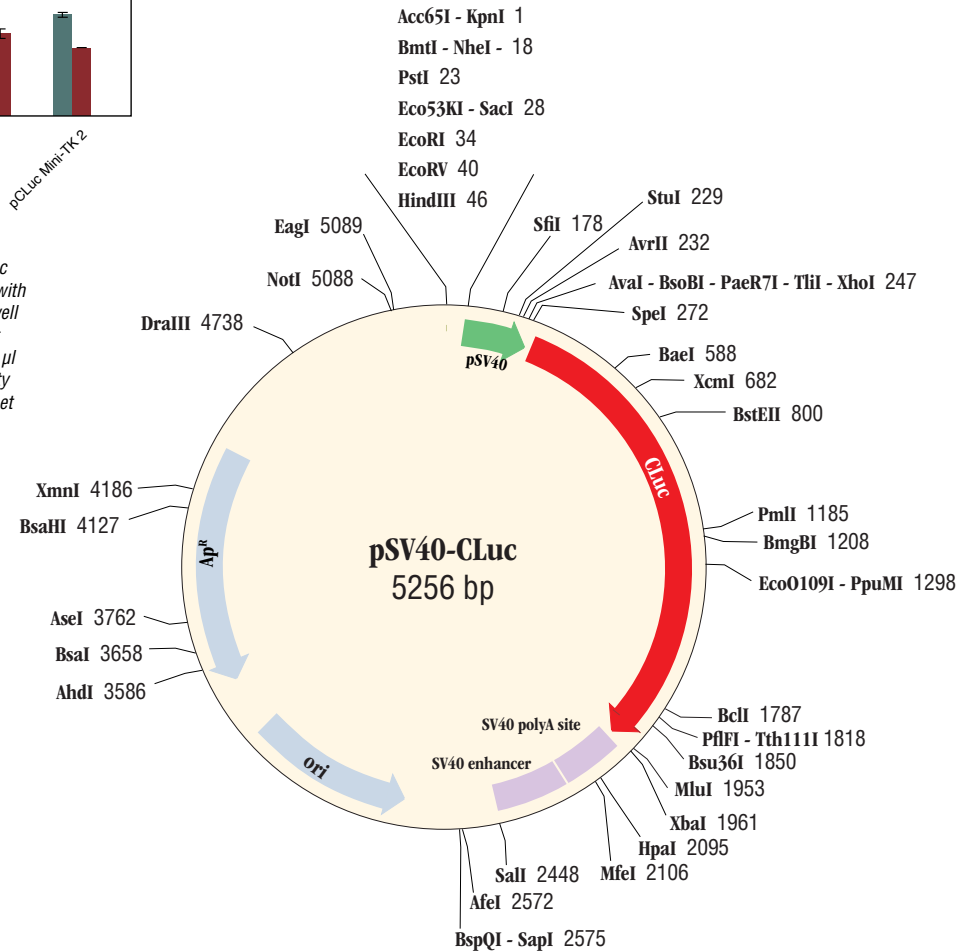
Figure 1: *Cypridina* Luciferase (CLuc) activity obtained from different CLuc plasmids. HeLa cell supernatants (20 µl) and lysates (5 µl) were assayed with the BioLux CLuc Assay Kit (NEB #E3309). HeLa cells were seeded in 12-well plates and transfected with 50 ng of CLuc-expressing plasmid per well. At 24 hr post-transfection, supernatants were collected and cell lysed in 100 µl per well using Luciferase Cell Lysis Buffer (NEB #B3321). The CLuc activity was measured in a Mithras LB940 (Berthold Technologies) luminometer set to: 50 µl of injection, 2 seconds of delay and 2 seconds of integration.

Applications:

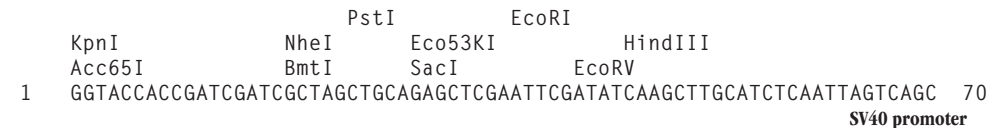
- The pSV40-CLuc Control Plasmid can be used as a control for assessing the efficiency of transfection in mammalian cells. Plasmids containing other constitutive promoter elements are also available (see Companion Products Sold Separately). CLuc can be used as a stand alone reporter or in conjunction 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.

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



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/



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)

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'-GTAAGGACAGTCTGGCAATGAAC-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 *Gaussia* Luciferase Assay Kit
#E3300S 100 assays
#E3300L 1,000 assays

BioLux *Gaussia* 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 BioLux *Cypridina* Luciferase Assay Kit, or associated assay reagents, in human diagnosis and measurement in relation to human health, contact busdev@neb.com.