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.

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/

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.

<table>
<thead>
<tr>
<th>Sample (µl)</th>
<th>Untransfected Lysate</th>
<th>pCLuc-Basic</th>
<th>pSV40-CLuc</th>
<th>pCMV-CLuc</th>
<th>2pTK-CLuc</th>
<th>pCLuc Mini-TK</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CLuc only</td>
<td>100,000</td>
<td>10,000</td>
<td>1,000</td>
<td>100</td>
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<tr>
<td>Assay solution only</td>
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<td>10,000</td>
<td>1,000</td>
<td>100</td>
<td>1</td>
<td>1</td>
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</table>

<table>
<thead>
<tr>
<th>Sample (µl)</th>
<th>Supernatant</th>
<th>Lysate</th>
<th>pCLuc-Basic</th>
<th>pSV40-CLuc</th>
<th>pCMV-CLuc</th>
<th>2pTK-CLuc</th>
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<tr>
<td>Assay solution only</td>
<td>100,000</td>
<td>10,000</td>
<td>1,000</td>
<td>100</td>
<td>1</td>
<td>1</td>
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</tbody>
</table>

For more information, 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´-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 NeoR marker for selection in mammalian cells.

Can I transfet 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® Cypridina Luciferase 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:

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
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