pCLuc-Basic 2 Vector

1-800-632-7799
info@neb.com
www.neb.com

N0317S 002111014100

N0317S

20 µg	**Lot: 0021110**	**Exp: 10/14**
0.5 µg/µl	**Store at –20°C**	

Description: pCLuc-Basic 2 is a cloning vector for expression in mammalian cells, containing a reporter gene but lacking promoter elements. The reporter gene is the secreted luciferase from the Ostracod *Cypridina noctiluca*. *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. The pCLuc-Basic 2 Vector contains a multiple cloning site (MCS) upstream of the CLuc coding sequence. 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 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* and *Gaussia*). Therefore, it is possible to assay both CLuc and GLuc independently in cell culture medium from cells expressing both reporters.

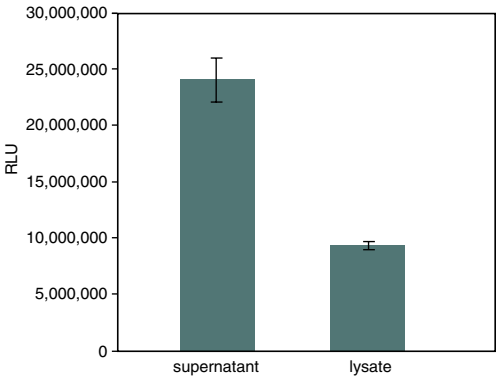


Figure 1: *Activity of Cypridina Luciferase in supernatants and lysates from a stable CLuc-expressing cell line. CLuc activity was measured from 20 µl of cell culture supernatant (500 µl total culture volume) and from 20 µl of cell lysate (100 µl total lysate volume).*

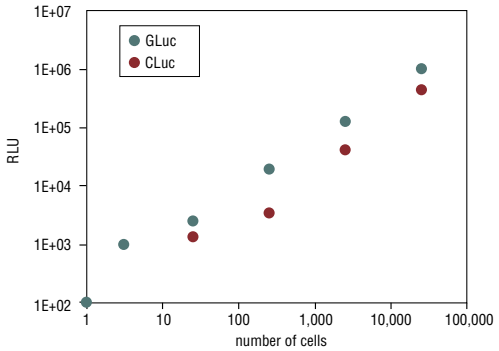
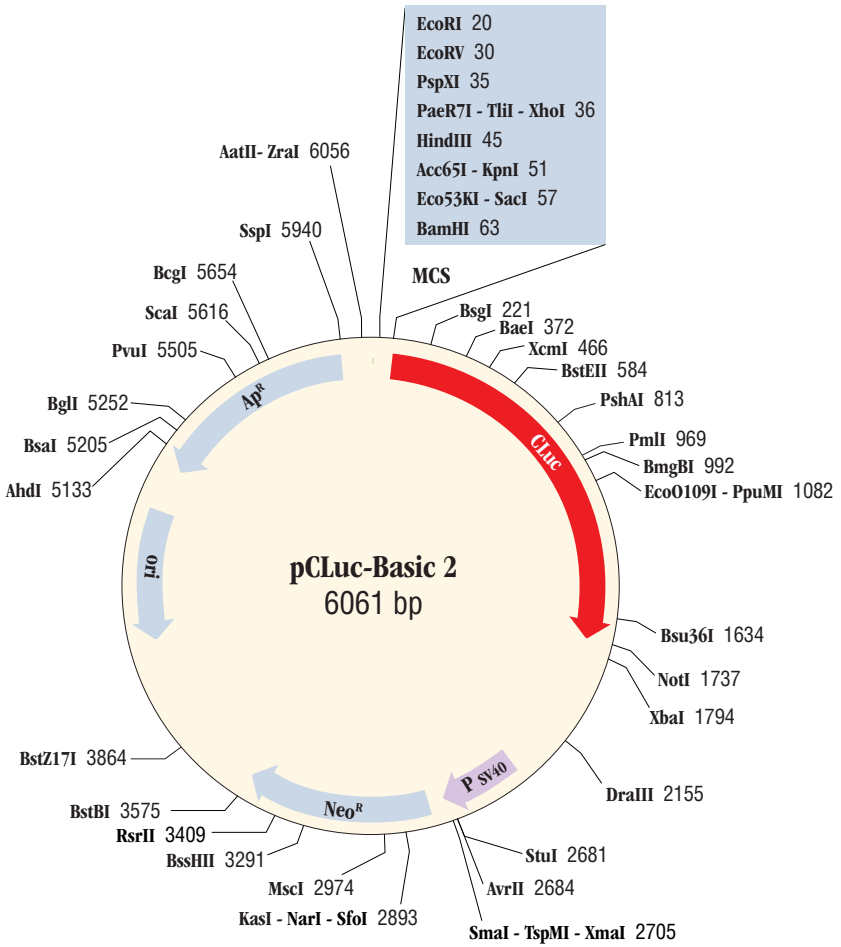


Figure 2: *The high sensitivity of both the CLuc and GLuc assays allows detection of very small numbers of cells expressing each protein. 20 µl of culture supernatant from the indicated number of cells expressing each reporter were assayed.*

- The pCLuc-Basic 2 Vector can be transfected into cells using any standard transfection protocol and stable cell lines can be established using Neomycin selection.

Applications:

- The pCLuc-Basic 2 Vector can be used to test promoters by cloning promoter element of interest into the MCS upstream of the CLuc reporter gene. For constitutive expression of CLuc, vectors containing promoters are 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 (2).



Restriction map of pCLuc-Basic 2 Vector 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/

```
                          EcoRI     EcoRV  PspXI   HindIII
  1   GACGGATCGGGAGATCTTGGAATTCTGCAGATATCCTCGAGCCCAAGCTT   50
                                          XhoI
                                          TliI
        Eco53KI                           PaeR7I
      KpnI  SacI  BamHI
 51   GGTACCGAGCTCGGATCCGCCACCATGAAGACCTTAATTCTTGCCGTTGC  100
      Acc65I                    M  K  T  L  I  L  A  V  A...
                                CLuc
```

pCLuc-Basic 2 multiple cloning site (MCS). The Cypridina Luciferase sequence is shown with a blue background. Only unique restriction sites are shown.

(see other side)

CERTIFICATE OF ANALYSIS

Features of pCLuc-Basic 2 Vector:

- Polylinker MCS: 20–68
- Start codon of CLuc: 75–77
  Stop codon: 1734–1736
- Signal peptide: 75–128
- Synthetic poly-A site: 1745–1793
- Neo promoter (SV 40): 2379–2714
- Neomycin resistance gene: 2766–3560
- Bacterial replication ori (pMB1): 4894–4306
- Amp resistance: 5925–5065

Recommended sequencing primers for pCLuc-Basic 2

Upstream of MCS:
pGLuc-Basic Forward Sequencing Primer (23-mer) (NEB #S1282) 5´-GGGGTTCCGCGCA-CATTTCCCCG-3´(6020–6042)

pBasic Reverse Primer (25-mer) (not available from NEB) 5´-TCAGAAGCCATAGAGCCCACCG-CAT-3´(1888–1864)

CLuc 3´ End Forward Primer (23-mer) (not available from NEB) 5´-GAGTTCAAGAAAGAATGCTA-CAT-3´(1671–1693)

CLuc 5´ End Reverse Primer (24-mer) (not available from NEB) 5´-GTAAGGACAGTCCTGGCAAT-GAAC-3´(143–120)

Frequently Asked Questions:

Where can I find the sequence of this plasmid?

The sequences of all the vectors sold by NEB are available online at www.neb.com.

Can I make a stable cell line with pCLuc-Basic 2?

Yes. One will need to use Neomycin selection (G418) after transfection.

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

pSV40-CLuc Vector
#N0318S 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 Control Plasmid
#N8081S 20 µg

pGLuc Basic Vector
#N8082S 20 µg

pTK-GLuc Vector
#N8084S 20 µg

pGLuc Mini-TK Vector
#N8086S 20 µg

TransPass™ D1 Transfection Reagent
#M2553S 0.5 ml

TransPass™ D2 Transfection Reagent
#M2554S 0.5 ml