pCLuc Mini-TK 2 Vector

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N0319S 002111014100

N0319S

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

Description: pCLuc Mini-TK 2 is a cloning vector for mammalian cells, containing a minimal promoter fragment from the HSV thymidine kinase (TK) promoter adjacent to a reporter gene, 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 of mammalian cells expressing the reporter gene. The pCLuc Mini-TK 2 Vector contains a MCS upstream of the minimal TK promoter for cloning promoter or enhancer elements. 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 *E. coli* strain ER2272 by a 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 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 pCLuc Mini-TK 2 Vector can be transfected into cells using any standard transfection protocol.

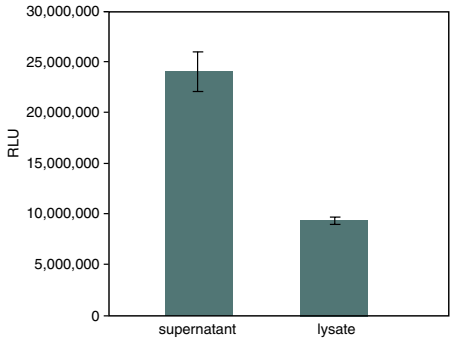


Figure 1: *Activity of Cypridina Luciferase in supernatants and lysates from a stable CLuc-expressing cell line. CLuc activity was measured from 20 µl of cell culture supernatant (500 µl total culture volume) and from 20 µl of cell lysate (100 µl total lysate volume).*

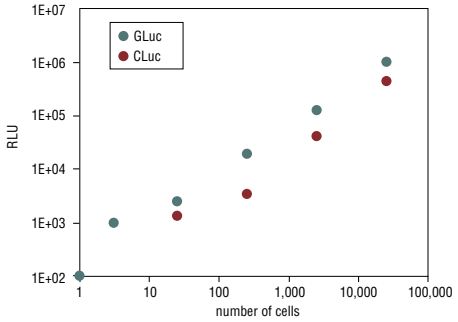


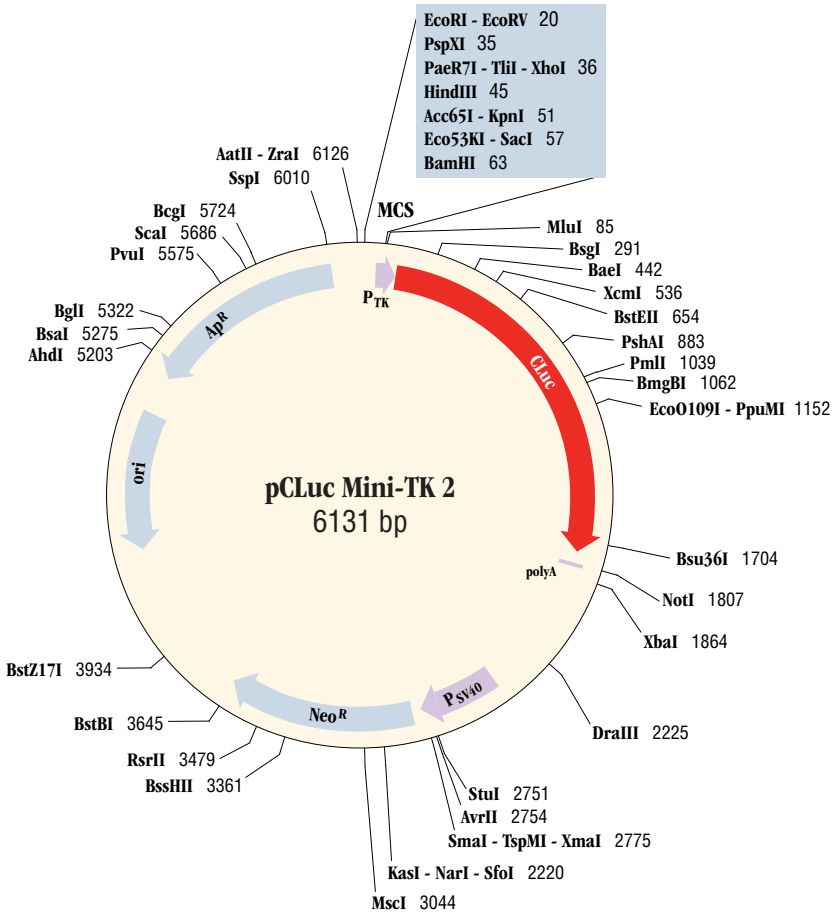
Figure 2: *The high sensitivity of both the CLuc and GLuc assays allows detection of very small numbers of cells expressing each protein. 20 µl of culture supernatant from the indicated number of cells expressing each reporter were assayed.*

Applications:

- The pCLuc Mini-TK 2 Vector can be used to test promoter or enhancer elements by cloning into the MCS upstream of the minimal TK promoter. For constitutive expression of CLuc, vectors containing constitutive promoter elements are available (see Companion Products Sold Separately).

Features of pCLuc Mini-TK 2 Vector:

- Polylinker upstream of Mini-TK: 20–68
- Minimal promoter from HSV-Thymidine Kinase (MiniTK): 69–137
- Kozak Consensus: 139–148
- CLuc ORF: 145–1806
- Start codon of CLuc 145–147
- Stop codon: 1804–1806
- Signal peptide: 145–198
- Synthetic poly-A site: 1815–1863



Restriction map of pCLuc-mini-TK 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/restriction_enzymes/dna_sequences_maps.asp.

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                          EcoRI     EcoRV   XhoI     HindIII
  1  GACGGATCGG GAGATCTTGG AATTCTGCAG ATATCCTCGA GCCCAAGCTT       50

       KpnI  SacI  BamHI               Mini-TK
 51  GGTACCGAGC TCGGATCCTT CGCATATTAA GGTGACGCGT GTGGCCTCGA       100

                                              Kozak
101  ACACCGAGCG ACCCTGCAGC GACCCGCTTA AAAGATC CGCCACC ATG AAG ACC 153
                                                  M   K   T
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pCLuc-mini-TK 2 multiple cloning site (MCS). Mini-TK promoter element is in pink; the Cypridina Luciferase sequence is shown with a red background.

Features of pCLuc Mini-TK 2 Vector (cont.):

- SV40 promoter (Neo^R): 2449–2784
- Neo^R Orf: 2836–3630
- SV40 early polyadenylation signal: 3804–3934
- Ori: pMB1 origin of replication (complement):4376–4964
- Amp^R (beta-lactamase complement): 5135–5995
- Two restriction sites are available for cloning elements downstream of the CLuc coding region. The NotI site is upstream of the polyA site and allows cloning of sequences which will become part of the CLuc mRNA. The XbaI site is downstream of the polyA site, sequences cloned into the XbaI site will not be incorporated into CLuc mRNA.
- All pLuc-2 vectors have improved poly-adenylation-transcription termination of the luciferase transcript. The polyadenylation signal is a synthetic polyadenylation sequence based on the β-globin gene (5).

Recommended sequencing primers for pCLuc Mini-TK 2 Vector (not available from NEB)

Upstream of MCS:
pGLuc Basic Forward Sequencing Primer (23-mer)
GGGGTTCCGCGCACATTTCCCCG (6090–6112)

pBasic Reverse Primer (25-mer)
TCAGAAGCCATAGAGCCCACCGCAT (1958–1934)

CLuc 3´ end Forward Primer (23-mer)
GAGTTCAAGAAAGAATGCTACAT (1741–1763)

CLuc 5´ End Reverse Primer (24-mer)
GTAAGGACAGTCCTGGCAATGAAC (213–190)

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: http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/dna_sequences_maps.asp.

Can I make a stable cell line with pCLuc Mini-TK 2 Vector?

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 than the ones used by *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 that produce high intensity bioluminescent signals. They oxidize different substrates that do not cross-react with each other. Therefore, *Cypridina* and *Gaussia* are an ideal pair for co-transfecting mammalian cells (2,3). Refer to the BioLux *Gaussia* Luciferase (GLuc) Assay Kits and GLuc expression vectors for more information.

References:

1. Nakajima, et al. (2004) *Biosci. Biotechnol. Biochem.,* 68, 565–570.
2. Otsuji, et al. (2004) *Anal. Biochemistry*, 329, 230–237.
3. Wu, et al. (2007) *Biotechniques*, 42, 290–292.

Companion Products Sold Separately:

BioLux® *Cypridina* Luciferase Assay Kit
#E3309S 100 assays
#E3309L 1,000 assays

pCMV-CLuc 2 Control Plasmid
#N0321S 20 µg

pCLuc-Basic 2 Vector
#N0317S 20 µg

pTK-CLuc Vector
#N0322S 20 µg

pSV40-CLuc Control Plasmid
#N0318S 20 µg

Luciferase Cell Lysis Buffer
B3321S 25 ml

BioLux® *Gaussia* Luciferase Assay Kit
#E3300S 100 assays
#E3300L 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