

pTK-CLuc Vector



N0322S

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

Description: The pTK-CLuc Vector is a mammalian expression vector that encodes the secreted luciferase from the Ostracod *Cypridina noctiluca* as a reporter, under the control of the constitutive HSV thymidine kinase 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). Because it is secreted CLuc can be detected in the culture medium of mammalian cells expressing the reporter gene. pTK-CLuc has a multiple cloning site (MCS) between the CLuc stop codon and the polyadenylation site. pTK-CLuc contains a selectable marker that is suited for creating stable integrants in the mammalian cell genome.

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 pTK-CLuc Vector can be transfected into cells using any standard transfection protocol.

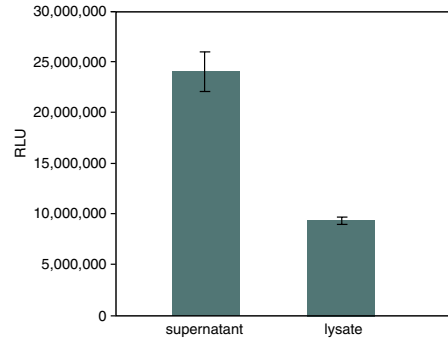


Figure 1: Activity of *Cypridina* Luciferase in supernatants and lysates from a stable CLuc-expressing cell line. CLuc activity was measured from 20 µl of cell culture supernatant (500 µl total culture volume) and from 20 µl of cell lysate (100 µl total lysate volume).

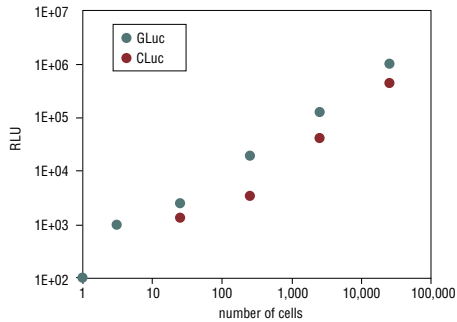
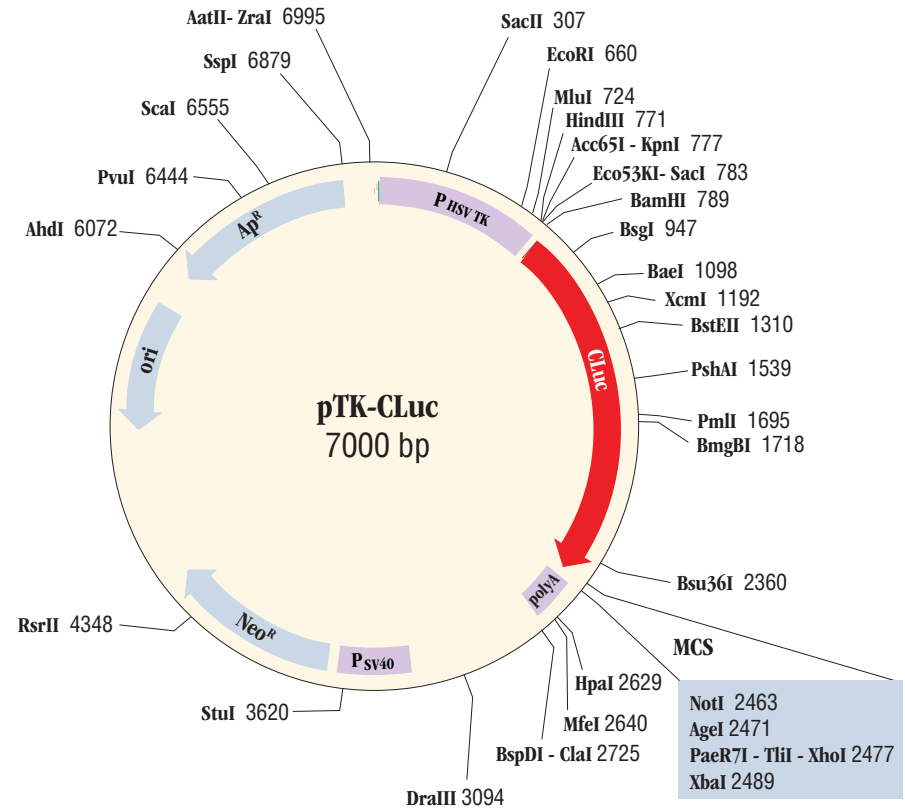


Figure 2: The high sensitivity of both the CLuc and GLuc assays allows detection of very small numbers of cells expressing each protein. 20 µl of culture supernatant from the indicated number of cells expressing each reporter were assayed.

Applications:

- The pTK-CLuc Vector can be used as a control for assessing the efficiency of transfection in mammalian cells. pTK-CLuc Vector has a multiple cloning site (MCS) between the CLuc stop codon and the polyadenylation signal. This allows the cloning of sequences that will be part of the CLuc mRNA, such as 3' UTR sequences, that can be used for RNA stability or RNAi or miRNA target evaluation.

Plasmids containing other constitutive promoter elements are also available (see Companion Products Sold Separately).



Restriction map of pTK-CLuc 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.

NotI AgeI XhoI XbaI
2463 GCGGCCGCACCGGTCTCGAGCATGCATCTAGA 2494

pTK-CLuc multiple cloning site (MCS).

Features of pTK-CLuc Vector:

- TK promoter (BgIII-HindIII): 18-776
- Start codon of CLuc: 801-803
- Stop codon: 2460-2462
- CLuc coding: 801-2462
- Signal peptide: 801-854
- Polylinker downstream of CLuc 2463-2489 NotI, AgeI, XhoI, XbaI
- Poly-A site (from SV40): 2490-2733
- NeoR promoter (SV40): 3318-3653
- NeoR: 3705-4499
- NeoR poly-A(SV40): 4673-4803
- Bacterial replication ori (pMB1) 5833-5245
- Amp Resistance gene 6864-6004
- At the 3' end of the CLuc ORF and past the STOP codon the NotI and XbaI restriction sites are available for cloning.

(see other side)

Recommended sequencing primers for pTK-CLuc

Vector (not available from NEB)

pBasic Reverse Primer (25-mer)

TCAGAAGCCATAGAGCCACCGCAT (2827-2803)

CLuc 3' end Forward Primer (23-mer)

GAGTTCAAGAAAGAATGCTACAT (2397-2419)

CLuc 5' End Reverse Primer (24-mer)

GTAAGGACAGTCTGGCAATGAAC (869-846)

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 pTK-CLuc 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

pCLuc Mini-TK 2 Vector

#N0319S 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

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U.S. Patent Nos. 7,718,389 and 7,989,621

U.S. Appln. Serial Nos. 12/588,671 and 13/067,565

Japanese Patent Nos. 4,761,150 and 4,484,429

Japanese Appln. Serial No.: 2006-280827; 2007-536587; 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.