pCMV-CLuc 2 Control Plasmid

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N0321S 006160919091

N0321S

20 µg **Lot: 0061609** **Exp: 9/19**

0.5 µg/µl **Store at –20°C**

Description: The pCMV-CLuc Control Plasmid is a mammalian expression vector that encodes the secreted luciferase from the Ostracod *Cypridina noctiluca* as a reporter, under the control of the constitutive CMV (cytomegalovirus) 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. 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 NEB10β 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 pCMV-CLuc 2 Control Plasmid can be transfected into cells using any standard transfection protocol.

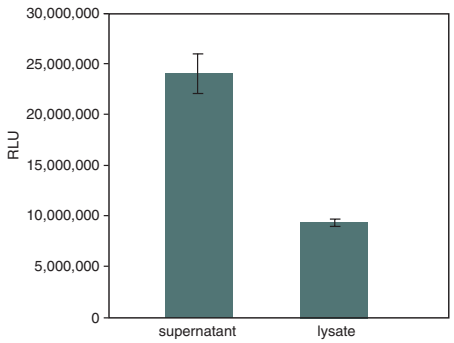


Figure 1: *Activity of Cypridina Luciferase in supernatants and lysates from a stable CLuc-expressing cell line. CLuc activity was measured from 20 µl of cell culture supernatant (500 µl total culture volume) and from 20 µl of cell lysate (100 µl total lysate volume).*

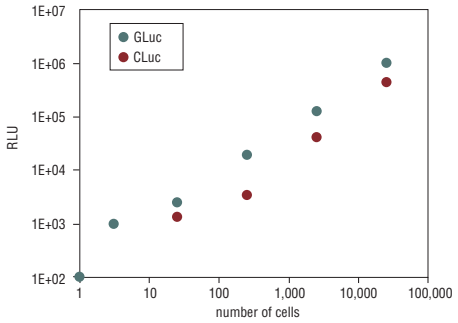


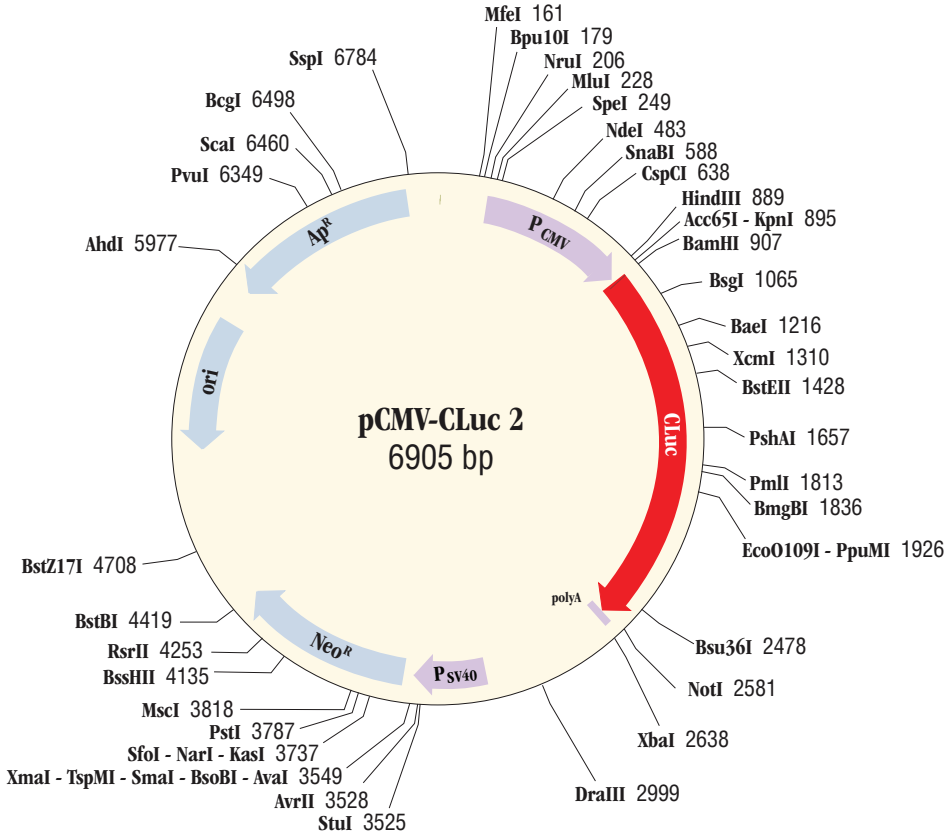
Figure 2: *The high sensitivity of both the CLuc and GLuc assays allows detection of very small numbers of cells expressing each protein. 20 µl of culture supernatant from the indicated number of cells expressing each reporter were assayed.*

Applications:

- The pCMV-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).

Features of pCMV-CLuc 2 Control Plasmid:

- CMV promoter: 209-863
- CLuc coding: 919-2580
- Start codon: 919-921
- Stop codon: 2578-2580
- Signal peptide: 919-972
- Synthetic poly-A site: 2589-2637
- Neo promoter (SV 40): 3223-3558
- Neomycin resistance gene: 3623-4404
- Bacterial replication ori (pMB1): 5738-5150



Restriction map of pCMV-CLuc 2 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/restriction_enzymes/dna_sequences_maps.asp.

- Amp resistance: 6769-5909
- All pLuc-2 vectors have improved polyadenylation-transcription termination of the luciferase transcript. The polyadenylation signal is a synthetic polyadenylation sequence based on the β-globin gene (4).

Recommended sequencing primers for pCMV-CLuc 2 Control Plasmid (not available from NEB)

T7 Universal Primer (20-mer)
TAATACGACTCACTATAGGG (863-882)

pBasic Reverse Primer (25-mer)
TCAGAAGCCATAGAGCCCACCGCAT (2732-2708)

CLuc 3´ End Forward Primer (23-mer)
GAGTTCAAGAAAGAATGCTACAT (2515-2537)

CLuc 5´ End Reverse Primer (24-mer)
GTAAGGACAGTCCTGGCAATGAAC (987-964)

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 pCMV-CLuc 2 Control Plasmid?

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

How do I assay for CLuc expression?

Please refer to the BioLux® CLuc Assay Kit (NEB #E3309).

(see other side)

CERTIFICATE OF ANALYSIS

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.
4. Levitt, et al. (1989) *Genes Dev.* 3, 1019–1025.

Companion Products Sold Separately:

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

pTK-CLuc Vector
#N0322S 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 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