pCMV-GLuc 2 Control Plasmid

**Description:** The pCMV-GLuc 2 Control Plasmid is a mammalian expression vector that encodes the secreted luciferase from the copepod *Gaussia princeps* as a reporter, under the control of the constitutive CMV (cytomegalovirus) promoter. *Gaussia* luciferase (GLuc) is a 19 kDa protein encoded by a "humanized" sequence, and it contains a native signal peptide at the N-terminus that allows it to be secreted from mammalian cells into the cell culture medium (1,2). A neomycin resistance gene (Neo R) allows it to be secreted from mammalian cells into the cell culture medium (1,2). A neomycin resistance gene: 2507–3301

**Advantages:**
- Multiple samples can be obtained from the same transfected cells (i.e., before and after experimental treatments or at multiple time points).
- GLuc is very stable in the cell culture medium (4°C for several days without any loss in activity). (see other side)
- GLuc does not use the same substrate as *Cypridina* luciferase. Therefore, it is possible to assay both GLuc and CLuc independently in cell culture medium from cells expressing both reporters (3,4).
- The pCMV-GLuc 2 Control Plasmid can be transfected into cells using any standard transfection and stable cell lines can be established using Neomycin selection.

**Applications:**
- The pCMV-GLuc 2 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).
- GLuc can be used as a stand alone reporter in conjunction with other compatible reporters such as *Cypridina* luciferase (CLuc) (3). GLuc and CLuc are ideally suited for co-expression as both are secreted and highly active enzymes providing ease of use and sensitivity (3,4).

**Features of pCMV-GLuc 2 Control Plasmid:**
- CMV promoter: 209–863
- GLuc coding: 920–1477
- Start codon: 920–922
- Stop codon: 1475–1477
- Signal peptide: 920–970
- Synthetic poly-A site: 1486–1534
- Neo promoter (SV40): 2120–2455
- Neomycin resistance gene: 2507–3301

**Recommended Sequencing Primers for pCMV-GLuc 2 Control Plasmid:**

- GLuc 5´ End Reverse Primer (24-mer)
  
- GLuc 3´ End Forward Primer (20-mer)

**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: https://www.neb.com/tools-and-resources/interactive-tools/dna-sequences-and-maps-tool.
- Can I generate a stable cell line with pCMV-GLuc 2 Control Plasmid? Yes. Selection for neomycin resistant colonies after transfection can be carried out by growing the cells in media containing G418.
- 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.

**Restriction map of pCMV-GLuc Vector. Only unique restriction sites are shown.** The complete sequence and restriction map is available at: http://www.neb.com/nebecomm/tech_reference/dna-sequences-and-maps-tool.

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How do I assay for GLuc expression?
Both the BioLux® Gaussia Luciferase Assay Kit (NEB #E3300) and the BioLux Gaussia Luciferase Flex Assay Kit (NEB #E3308) can be used to detect GLuc expression.

Is there another secreted reporter that can be used with GLuc?
Yes. Gaussia and Cypridina are both secreted luciferases, which produce high bioluminescent signal intensity. They oxidize different substrates that do not cross-react with each other. Therefore, Gaussia and Cypridina are an ideal duo for co-transfecting mammalian cells (2,3). Refer to the BioLux Cypridina Luciferase (CLuc) Assay Kits and CLuc expression vectors for more information.

References:

Companion Products Sold Separately:
- BioLux Gaussia Luciferase Assay Kit #E3300S 100 assays
  #E3300L 1,000 assays
- BioLux Gluc Flex Assay Kit #E3308S 100 assays
  #E3308L 1,000 assays
- Luciferase Cell Lysis Buffer #B3321S 25 ml
- pGLuc-Basic 2 Vector #N8082S 20 µg
- pGLuc Mini-TK 2 Vector #N8086S 20 µg
- pSV40-Gluc Control Plasmid #N0323S 20 µg
- pTK-Gluc Vector #N8084S 20 µg
- Anti-Gluc Antibody #N8023S 0.2 ml
- BioLux Cypridina Luciferase Assay Kit #E3309S 100 assays
  #E3309L 1,000 assays
- BioLux Cypridina Luciferase Starter Kit #E3314S 100 assays
  #E3314L 1,000 assays
- pCluc-Basic 2 Vector #N0317S 20 µg
- pCluc Mini-TK 2 Vector #N0324S 20 µg
- pCMV-CLuc 2 Control Plasmid #N0321S 20 µg
- pSV40-CLuc Control Plasmid #N0318S 20 µg
- pTK-CLuc Vector #N0322S 20 µg

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