Certificate of Analysis

pGL4.50[*luc2*/CMV/Hygro] Vector:

Part No.	Size
E131A	20µg

Instructions for use of this product can be found in the pGL4 Luciferase Reporter Vectors Technical Manual #TM259, available online at: **www.promega.com/protocols**

Part# 9PIE131
Revised 4/13

AF9PI E131 0413E131

Description: The pGL4.50[*luc2*/CMV/Hygro] Vector[a-f] (Cat.# E1310) encodes the luciferase reporter gene *luc2* (*Photinus pyralis*), which has been codon optimized for mammalian expression. This vector also is engineered with fewer consensus regulatory sequences for reduced backgrounds and a decreased risk of anomalous transcription.

This vector contains the following features:

- *luc2* reporter gene for expression in mammalian cells
- CMV promoter for high translational expression
- SV40 late poly(A) signal sequence is positioned downstream of *luc2* to provide efficient transcription termination and mRNA polyadenylation
- Binding region for RV primer 3 and RV primer 4
- Synthetic poly(A) signal/transcription start site
- Synthetic Hygromycin B-resistance gene for mammalian cell selection of the plasmid
- Plasmid replication origin
- *Amp*^{r} gene for bacterial selection for vector amplification

For more information, see the *pGL4 Luciferase Reporter Vectors Technical Manual* #TM259, available online at: www.promega.com/protocols

Concentration: 1µg/µl.

GenBank® Accession Number: EU921840.

Storage Buffer: The pGL4.50[*luc2*/CMV/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See the Product Information Label for storage temperature recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See the label for expiration date.

Usage Note:

Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in a specified sample of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of this vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: A_{260}/A_{280} >1.80; A_{260}/A_{250} >1.05.

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: www.promega.com/vectors/

Restriction Digestion: The functional purity of this vector DNA is verified by successful incubation with a variety of restriction enzymes at 37°C for one hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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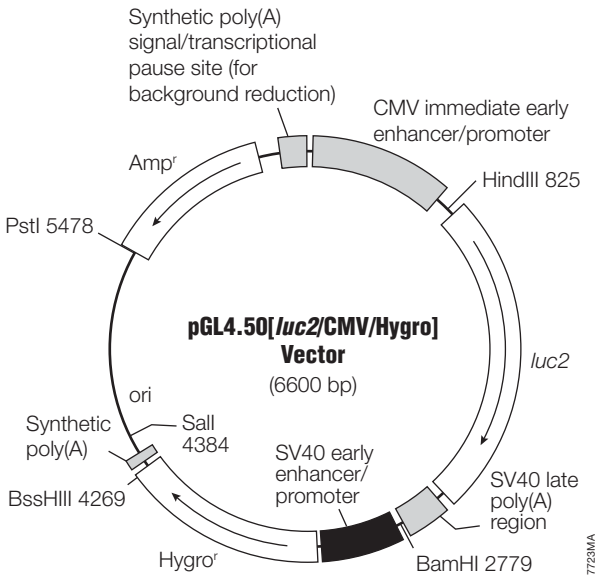
Promega

Features list and map for the pGL4.50[*luc2*/CMV/Hygro] Vector

CMV immediate early enhancer/promoter	14–755
luc2	859–2511
SV40 late poly(A) region	2546–2767
SV40 early enhancer/promoter	2815–3233
Synthetic hygromycin coding region (Hyg^r)	3258–4295
Synthetic poly(A)	4319–4367
Reporter vector primer 4 binding region	4434–4453
Replication origin	4691
Synthetic beta-lactamase (Amp^r) coding region	5482–6342
Synthetic poly(A) signal/transcriptional pause region	6447–6600
Reporter vector primer 3 binding region	6549–6568



Provided in cooperation with Xenogen Corporation and Caliper Life Sciences for use in in vivo bioluminescence imaging applications.

XENOGEN now part of Caliper Life Sciences

Caliper LifeSciences

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[c]U.S. Pat. No. 5,670,356.

[d]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

[e]U.S. Pat. No. 7,728,118.

[f]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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