Certificate of Analysis

pGL4.50[/uc2/CMV/Hygro] Vector:

Part No. Size E131A 20µq



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

Description: The pGL4.50[/uc2/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[/uc2/CMV/Hygro] Vector is supplied in 10mM Tris-HCI (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.

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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 (Hygr)	3258-4295
Synthetic poly(A)	4319-4367
Reporter vector primer 4 binding region	4434-4453
Replication origin	4691
Synthetic beta-lactamase (Ampr) coding region	5482-6342
Synthetic poly(A) signal/transcriptional pause region	6447-6600
Reporter vector primer 3 binding region	6549-6568

Synthetic poly(A) signal/transcriptional pause site (for background reduction) CMV immediate early enhancer/promoter Amp HindIII 825 Pstl 5478 pGL4.50[/uc2/CMV/Hygro] Vector luc2 (6600 bp) Synthetic Sall 4384 poly(A) SV40 early enhancer/ SV40 late BssHIII 4269 promoter poly(A) region Hygro^r BamHI 2779

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



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