Certificate of Analysis

pGL4.77[*hRlucP*/Hygro] Vector:

Part No.	Size
E695A	20µg

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

Part# 9PIE695
Revised 8/13

Description: The pGL4.77[*hRlucP*/Hygro] Vector[a-d] encodes the luciferase reporter gene *hRlucP* (*Renilla reniformis*) and is designed for high expression and reduced anomalous transcription. This vector also contains a mammalian selectable marker for hygromycin resistance in which the number of transcription factor binding sites has been reduced and mammalian codon usage optimized. The pGL4 Vectors are engineered with fewer consensus regulatory sequences than the pGL3 Vectors and a synthetic reporter gene that has been codon optimized for mammalian expression.

The pGL4.77[*hRlucP*/Hygro] Vector is a basic vector with no promoter. However, it contains a multiple cloning region that allows cloning of a promoter of choice. The *hRlucP* reporter gene contains hPEST, a protein destabilization sequence. The protein encoded by *hRlucP* responds more quickly and with greater magnitude to changes in transcriptional activity than the *hRluc* gene, its more stable counterpart.

Concentration: 1µg/µl.

GenBank® Accession Number: AY8469320.

Storage Buffer: The pGL4.77[*hRlucP*/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 expiration date on the product information label.

Usage Notes:

1. For easy transfer from one pGL4 Vector to another, the multiple cloning region is consistent throughout the pGL4 Vector series. For easy transfer between pGL3 Vectors and pGL4 Vectors, many of the restriction enzyme sites present in the pGL3 Vectors are also present in the pGL4 Vectors.
2. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assays

Nuclease Assay: Following incubation of 1µg of pGL4.77[*hRlucP*/Hygro] Vector in standard restriction digest buffers at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$ at pH 7.4.

Sequence: The pGL4.77[*hRlucP*/Hygro] Vector has been completely sequenced and is 100% identical to the published sequence, available at: **www.promega.com/vectors**

Signed by: J. Stevens

J. Stevens, Quality Assurance

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[b]Patent pending.

[c]U.S. Pat. No. 7,906,282 and European Pat. No. 1341808.

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

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pGL4.77[*hRlucP*/Hygro] Vector Features and Maps

The following features are present in the vector based on nucleotide sequence.

Multiple cloning region	1–70
hRlucP reporter gene	100–1158
SV40 late poly(A) signal	1198–1419
SV40 early enhancer/promoter	1467–1885
Synthetic hygromycin (Hyg^r) coding region	1910–2947
Synthetic poly(A) signal	2971–3019
Reporter Vector primer 4 (RVprimer4) binding region	3086–3105
*Col*EI-derived plasmid replication origin	3343
Synthetic β-lactamase (Amp^r) coding region	4134–4994
Synthetic poly(A) signal/transcriptional pause site	5099–5252
Reporter Vector primer 3 (RVprimer3) binding region	5201–5220

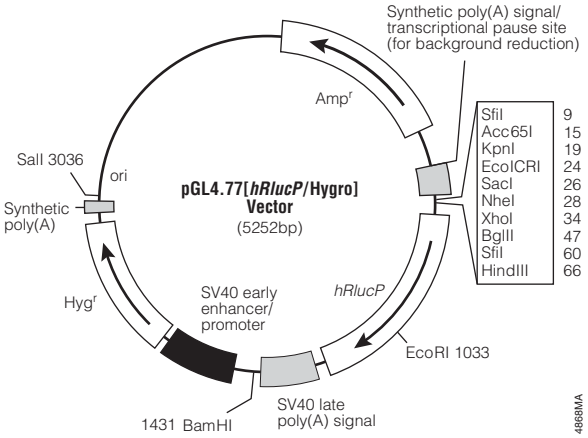

Figure 1. pGL4.77[*hRlucP*/Hygro] Vector map.

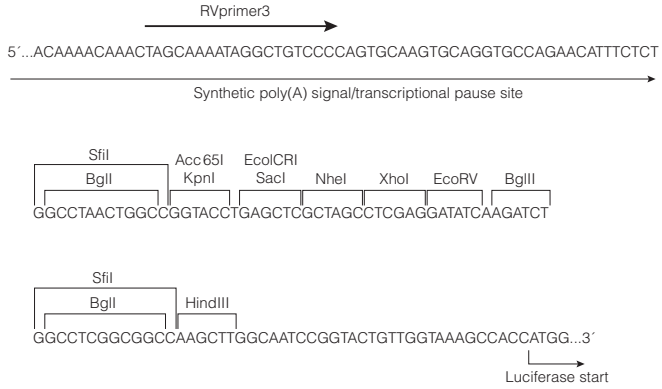

Figure 2. Multiple cloning region of the pGL4.77[*hRlucP*/Hygro] Vector.

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