Genlantis

A division of Gene Therapy Systems, Inc.

gWIZ Secreted Alkaline Phosphatase (SEAP) Mammalian Expression Vector

PRODUCT SUMMARY

Cat. No: P050200

Description: gWIZ vectors represent a new series of plasmids that have been engineered to produce the highest levels of transgene expression in a wide range of mammalian cells and tissues. It contains a proprietarily modified promoter followed by the intron A from the human cytomegalovirus (CMV) immediate early gene and a high-efficiency artificial transcription terminator. The expression vector is constructed in the context of a plasmid backbone extensively modified to achieve the enhanced levels of trangene expression in mammalian cells as well as high efficiency of plasmid production in *E. coli*.

Components: 25 µg gWIZ Secreted AP plasmid in 25 µl sterile TE buffer.

Storage: Store at -20°C.

Comments: gWIZ is suitable for in vitro and in vivo gene expression studies and applications. Use Kanamycin as selection to grow the plasmid in *E. coli*.

INTRODUCTION

The CMV immediate early gene (IE) promoter/enhancer is the most widely used constitutive promoter for expressing high levels of trangene product in many mammalian cells and tissues. However, not all CMV IE gene promoter/enhancer-based expression vectors are created equal. Depending on the actual CMV IE gene sequences used and the context of the plasmid backbone upon which the expression cassette is constructed, the expression levels can vary as much as two orders of magnitude. The CMV IE promoter sequences contained in the gWIZ vectors have been systemically modified to remove those sequences that are redundant and deleterious to the high levels of expression while retaining those sequences that are of high transcriptional potency. After coupling the modified promoter with a high-efficiency synthetic transcriptional terminator, the whole expression cassette is finally constructed on a plasmid backbone that has also been streamlined and modified to accommodate the highest levels of expression in mammalian cells as well as high yield of plasmid production in *E. coli*. The resulting plasmid, gWIZ expression vector, is capable of fully unleashing the potential of the CMV promoter and giving the highest levels of expression possible both *in vitro* and *in vivo*.

USAGE

- For extremely high levels of transgene expression in mammalian cells and tissues
- Can be used with GenePORTER® 2 (Cat. # T202007 or T202015) to transfect a wide variety of mammalian cells and tissues

DETECTION OF THE EXPRESSED GENE

Secreted alkaline phosphatase: 48 hours post transfection, supernatants from transfected cells are heated at 65°C for 30 minutes to inactivate endogenous alkaline phosphatase activity. The SEAP activity is quantitatively determined by using a colorimetric assay based on hydrolysis of the chromogenic substrate para-nitrophenyl phosphate (PNPP). 1 mg/ml of PNPP reagent is prepared in 1mM MgCl2, 1M Diethanolamine, pH 9.8. Into each well of a 96-well plate, 10 ul of 0.05% Zwittergent in PBS (Ca^{++} and Mg^{++} free) is added, and then mixed with 20 ul of the heated cell culture media. For control wells, 20 ul of water is used to normalize the volume. An alkaline phosphatase (AP) standard (EIA grade calf intestine alkaline phosphatase) can be used to generate a standard curve from 1 to 100 pg per well. To start the enzymatic reaction, 200ul of the PNPP substrate is added to each well. The reaction is allowed to stand at room temperature for 5-45minutes prior to analysis. The use of 0.05% Zwittergent in PBS as the diluent virtually reduces the background to zero, which increases the sensitivity of the assay. The plates are read at 405nm using either kinetic or static settings.

RELATED PRODUCTS

Product	Cat. Nos.
gWIZ Blank	P000200
gWIZ β-gal	P010200
gWIZ CAT	P020200
gWIZ Luciferase	P030200
gWIZ GFP	P040400
GenePORTER® 2 Transfection Reagent	
75 transfections (0.75 ml)	T202007
150 transfections (1.5 ml)	T202015

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