pGL4.29[luc2P/CRE/Hygro] Vector:

Part No. 9PIE847 20µg

Description: The pGL4.29[luc2P/CRE/Hygro] Vector (a–f) contains a cAMP response element (CRE) that drives the transcription of the luciferase reporter gene luc2P (Photinus pyralis). luc2P is a synthetically-derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription. The luc2P gene contains hPest, a protein destabilization sequence. The protein encoded by luc2P responds more quickly than the protein encoded by the lux2 gene upon induction. The vector backbone contains an ampicillin resistance gene to allow selection in E. coli and a mammalian selectable marker for hygromycin resistance.

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NAUCUSE ASSAYS

Nuclease Assay: Following incubation of 1µg of the vector in restriction digest buffer B at 37°C for 16 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 pGL4.29[luc2P/CRE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: www.promega.com/vectors
Day 1: Plate Cells
1. Grow HEK 293 cells in DMEM/FBS to approximately 75% confluency.
2. Harvest cells via trypsinization. Remove the DMEM/FBS, wash the cells with DPBS and add the trypsin/DPBS (1X volume). After 2 minutes, add a 4X volume of DMEM/FBS, collect the cell suspension and pellet the cells by centrifugation. Aspirate the supernatant and resuspend in DMEM/FBS at a concentration of 10,000 viable cells/90µl DMEM/FBS.
3. Dispense 90µl of the cell suspension into the wells of a 96-well plate. Plate enough wells to perform each test condition in triplicate.
4. Cover the plate and place it in a tissue culture incubator at 37°C overnight (or for 24 hours).

Day 2: Transfect Cells
1. Prepare the DNA transfection master mix. Each well of 96-well plate to be transfected requires 10µl DMEM, 0.3µl TransIT®-LT1 and 0.1µg pGL4.29[luc2P/CRE/Hygro] plasmid DNA. To prepare the master mix, calculate the total number of wells that will be transfected and prepare 110% of this amount. It is recommended that at least 10 wells of master mix be prepared.
   • For each well, mix 10µl DMEM and 0.3µl TransIT®-LT1 in a microcentrifuge tube, briefly vortex at maximum setting, and incubate at room temperature for 15 minutes.
   • For each well to be transfected, add 0.1µg of pGL4.29[luc2P/CRE/Hygro] vector to the DMEM/TransIT®-LT1, vortex briefly and incubate at room temperature for 15 minutes.
2. Add 10µl of master mix to each well that is to be transfected.
3. Cover the plate and place it in a tissue culture incubator at 37°C overnight (or for 24 hours).

Day 3: Induce Transfected Cells and Measure Luciferase Activity
1. Prepare 10X induction and 10X control solutions. Calculate the volume of 10X induction and 10X control solution by multiplying the number of wells needed for each solution by 11µl and prepare 110% of this amount.
   • 10X induction solution: Dilute 100mM stock forskolin solution to 1mM (1:100) in DMEM.
   • 10X control solution: Dilute DMSO 1:100 in DMEM.
2. Add 11µl of 10X induction solution to the cells to be induced and 11µl of 10X control solution to the control noninduced cells.
3. Return the plate to the tissue culture incubator and induce for 5 hours.
5. Calculate the fold induction as follows:
   Fold Induction = Average relative light units of induced cells
                   Average relative light units of control cells