pSELECT-hygro-mcs
Dual expression cassette plasmid for the expression of one gene of interest
Catalog code: pseth-mcs
https://www.invivogen.com/pselect-hygro
For research use only
Version 19A21-MM

PRODUCT INFORMATION
Contents
- 20 µg of pSELECT-hygro-mcs plasmid provided as lyophilized DNA
- 1 ml of Hygromycin B Gold at 100 mg/ml

Storage and stability
- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store Hygromycin B Gold at 4°C or -20°C. The expiry date is specified on the product label.

Quality control
- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE
pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both E. coli and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human β-globin polyA terminates the transcription of the selectable marker.

pSELECT-hygro-mcs contains a multiple cloning site (MCS) downstream of the composite promoter for convenient cloning of a gene of interest.

METHODS
Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:
Plasmid amplification and cloning can be performed in E. coli GT116 or other commonly used laboratory E. coli strains, such as DH5α.

Hygromycin B usage:
This antibiotic can be used for E. coli at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

PLASMID FEATURES
First expression cassette
- hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene in vivo. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- MCS: The multiple cloning site contains the following restriction sites: 5' - Sal I, SgrA I, BamH I, Eco47 III, Nco I, Nhe I - 3'
- SV40 pAn: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- ori: a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.

Second expression cassette
- CMV enh/prom: The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.
- Hygro: Resistance to Hygromycin B is conferred by the hph gene from E. coli which encodes a phosphotransferase. The hph gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and E. coli.
- βGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

References
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(3821 bp)