pVITRO2-blasti-mcs
A multigenic plasmid for high levels of expression
Catalog code: pvitro2-mcs
https://www.invivogen.com/pvitro2-mcs
For research use only
Version 20H17-MM

PRODUCT INFORMATION
Contents
- 20 µg of pVITRO2-blasti-mcs provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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
pVITRO is a family of plasmids developed mainly for in vitro studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

pVITRO2-blasti-mcs plasmid is selectable with blasticidin in both E. coli and mammalian cells. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

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.

Blasticidin usage:
Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

PLASMID FEATURES
- hFerH and hFerL composite promoters: Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5’ untranslated region (5’UTR) of the ferritin mRNAs, and the iron regulatory protein. To eliminate the iron regulation of the ferritin promoters, the 5’UTR of FerH and FerL have been replaced by the 5’UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- SV40 enhancer which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.
- CMV enhancer: The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer.
- SV40 pAn: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.
- pMB1 ori: a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.
- FMDV IRES: The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression.

Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:
Age I, Eco RV, Bam HI, Sal I and Avr II
- Age I is compatible with Bsp EI and Sgr Al.
- Eco RV (blunt-end restriction enzyme).
- Bam HI is compatible with Bgl II, Bst YI and Bcl I.
- Sal I is compatible with Ava I and Xho I.
- Avr II is compatible with Xba I, Spe I and Nhe I.

MCS2 contains the following restriction sites:
Sgr Al, Bgl II, Xho I and Nhe I
- Sgr Al is compatible with Bsp EI and Age I.
- Bgl II is compatible with Bam HI, Bst YI and Bcl I.
- Xho I is compatible with Ava I and Sal I.
- Nhe I is compatible with Xba I, Spe I and Avr II.


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