Product usage

Before using this product, please read the Limited Use statement below

Important Limited Use information for pUNO1-sACP5

The purchase of the pUNO1-sACP5 vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact us at outlicensing@invivogen.com.

pUNO1-sACP5

A secreted ACP5 Reporter Gene System selectable with Blasticidin

Catalog code: puno1-sacp5

https://www.invivogen.com/acp5-reporter-gene

For research use only

Version 240722-AK

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 1 x 1 ml Blasticidin at 10 mg/ml
- 0.5 ml of each QUANTI-Star™ reagent and QUANTI-Star™ buffer (sufficient to prepare 50 ml of the ACP5 detection reagent).

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year.
- 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 full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

• **Obtaining a gene to subclone into another vector.** The gene of interest is flanked by two unique restriction sites allowing its convenient excision. These restriction sites are compatible with other restriction sites contained in multiple cloning sites, thus facilitating subcloning.

• **Stable gene expression in mammalian cells.** pUNO1 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO1 plasmids contain the blasticidin-resistance gene (*bsr*) driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO1 allows high levels of expression and secretion (where applicable) of the gene product.

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* GT115 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. For *E. coli* GT115 or other commonly used laboratory *E. coli* strains, such as DH5α, we recommend using Blasticidin at 100 µg/ml. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

PLASMID FEATURES

• **hEF1-HTLV hybrid promoter** is a composite promoter comprised of the human Elongation Factor-1α (hEF-1α) core promoter[1] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[2] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **sACP5:** Also known as tartrate-resistant acid phosphatase (TRAP or TRAPase). ACP5 is a metalloprotein enzyme, usually located in the lysosome. The secreted (s)ACP5 features a signaling sequence, that - when expressed in mammalian or insect cells - mediates the export of sACP5 from within the cell to the surrounding culture medium. The level of ACP5 activity in the cell culture supernatant is easily quantified using QUANTI-Star™, our easy-to-use detection reagent. The gene is flanked by convenient cloning sites for easy subcloning (**5' end:** AgeI, NcoI. **3' end**: EcoR I site).

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[3].

• **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **hCMV enh-prom** are the human cytomegalovirus (hCMV) enhancer and promoter that drive the expression of the blasticidin resistance in mammalian cells.

• ***bsr* (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV enhancer-promoter in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **hβGlo pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference[4] and possible recombination events with the SV40 polyadenylation signal.

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91:217-23. **2. Takebe Y. *et al.*, 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **3. Carswell S. & Alwine JC., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(:4248-58. **4. Yu J. & Russell JE., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. Mol Cell Biol. 21:5879-88.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Star™	Detection reagent	rep-qst1
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT1155	Competent *E. coli*	gt115-11

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com