pUNO1-<Gene>-GFP
A plasmid expressing a TLR gene fused to a GFP gene
Catalog code: p<gene>-gfp
https://www.invivogen.com/trl-gfp-fusion
For research use only
Version 20A20-MM

PRODUCT INFORMATION

Contents
- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 mg/ml

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 at least for 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.
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GENERAL PRODUCT USE

pUNO1-TRL::GFP plasmids can be used directly for in vitro or in vivo transfection experiments. They are selectable with blasticidin, an antibiotic that allows the selection of stable mammalian clones in only a few days.

TLR::GFP fusion genes are under the control of a strong and ubiquitous composite promoter, called EF1α-HTLV, comprised of the elongation factor 1 alpha (EF-1α) core promoter and the R-U5' of the human T cell leukemia virus (HTLV).

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. For E. coli GT116 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

- **EF-1α-HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «housekeeping» 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 and segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat 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.
- **TLR::GFP fusion gene** was generated by fusing the C terminus of a TLR gene to a GFP variant. A synthetic intron was added between both moieties to increase the activity of GFP. This hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm). The TLR::GFP fusion gene is under the control of the EF1α/HTLV promoter.
- **SV40 pAn**: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA.
- **pMB1 ori** is a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV promoter & enhancer** drives 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 promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and E. coli transformants.
- **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of bsr. The use of beta-globin pAn minimizes interference and possible recombination events with the SV40 polyadenylation signal.


RELATED PRODUCTS

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<tr>
<th>Product</th>
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<tbody>
<tr>
<td>Blasticidin</td>
<td>Selection antibiotic</td>
<td>ant-bl-1</td>
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<tr>
<td>ChemiComp GT116</td>
<td>Competent E. coli</td>
<td>gt116-11</td>
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TECHNICAL SUPPORT

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