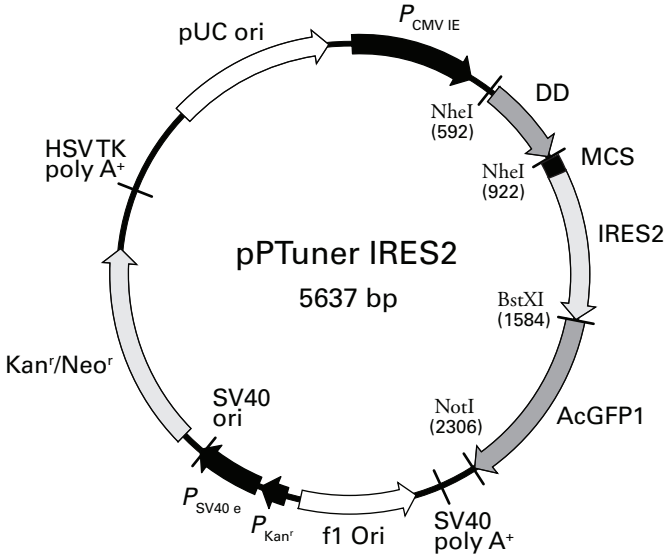
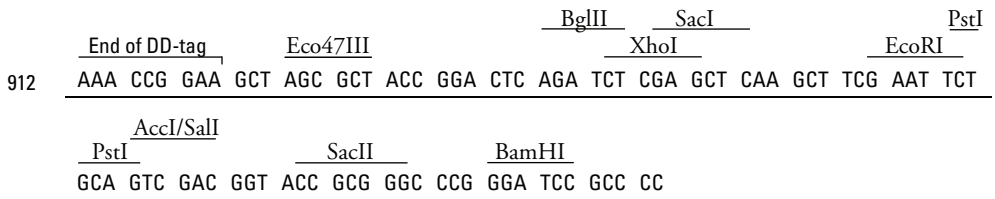
Vector Information

pPTuner IRES2 Vector Information PT4040-5

Catalog No. 632168





pPTuner IRES2 Vector Map and Multiple Cloning Site (MCS).

Description

pPTuner IRES2 is a bicistronic expression vector that allows you to precisely regulate the amount of your protein of interest in mammalian cells. The vector encodes a 12 kDa, FKBP (L106P) destabilization domain (DD; 1) that is expressed as an N-terminal tag on your protein of interest; this domain causes the rapid degradation of any protein to which it is fused. Once expressed, the amount of DD-tagged protein present in the cell can be rapidly increased by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeable molecule that binds to the DD tag, 'shielding' the fusion protein from proteasomal degradation. pPTuner IRES2 allows the simultaneous expression of your DD-tagged protein of interest and AcGFP1 fluorescent protein from the same bicistronic mRNA transcript. Because AcGFP1 is unaffected by the DD tag, it can be used as an indicator of transfection efficiency, as well as a marker for selection and cell sorting.

AcGFP1 is a human codon-optimized variant of the *Aequorea coerulescens* green fluorescent protein, AcGFP. Bicistronic expression of AcGFP1 and the DD-tagged protein of interest is facilitated by the encephalomyocarditis virus (EMCV) internal ribosome entry site 2 (IRES2). This IRES allows cap-independent translation of AcGFP1 from an internal start site at the IRES/AcGFP1 junction (2, 3). Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$) located just upstream of the DD tag sequence.

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR113809; published 17 January 2011)

pPTuner IRES2 contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. The vector also contains a neomycin/kanamycin resistance cassette that allows G418 selection of stably transfected eukaryotic cells (4). In addition, a bacterial promoter located upstream of this cassette allows kanamycin resistance in *E. coli*. The vector also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pPTuner IRES2 is available in the ProteoTuner Shield System N (w/ AcGFP1) [Cat. No. 632168]. It is designed to co-express your DD-tagged protein of interest and AcGFP1 in mammalian cells. In order to create your DD-tagged protein of interest, your gene of interest must be cloned into the MCS in the same reading frame as the DD tag sequence, and it must contain a stop codon at the end of its coding sequence. The simultaneous expression of AcGFP1 allows the selection of transfectants by flow cytometry (or other detection methods) with standard FITC filter sets (AcGFP1 has an excitation maximum of 475 nm and an emission maximum of 505 nm).

When cells expressing a DD-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD tag and protects the fusion protein from degradation. As a result, the protein quickly accumulates inside the cells in amounts that are directly proportional to the concentration of Shield1 in the medium. If the cells are subsequently grown in medium lacking Shield1, the DD tag is no longer stabilized, and the fusion protein is rapidly degraded. Because the effects of Shield1 are concentration-dependent and reversible, it is possible to fine-tune the amount of fusion protein present in the cells simply by adjusting the concentration of Shield1 in the medium (1).

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- DD (FKBP-L106P destabilization domain): 597–920
- MCS (multiple cloning site): 924–995
- IRES 2 (EMCV): 996–1580
- AcGFP1 (*Aequorea coerulescens* green fluorescent protein): 1584–2303
- SV40 poly A$^+$ signal: 2456–2506
- f1 origin of replication (allows the production of single-stranded DNA): 2553–3008 (complementary)
- P_{Kan^r} (Bacterial promoter for Kan^r gene expression): 3070–3105
- $P_{SV40\ e}$ (SV40 early promoter and enhancer): 3182–3411
- SV40 origin of replication: 3349–3484
- Kan^r/Neo^r (Tn5 kanamycin/neomycin resistance gene): 3533–4327
- HSV TK poly A$^+$ (herpes simples virus thymidine kinase poly A$^+$ signal): 4563–4581
- pUC origin of replication: 4912–5555

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418.

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells. Single-stranded DNA production requires a host containing an F plasmid, such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.
2. Jackson, R.J. *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
3. Jang, S.K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Notice to Purchaser
Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

AcGFP is covered by U.S. Patent No. 7,432,053.

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com. For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com or click here for more information.