

ProteoTuner™ Tag Kit

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Catalog No.

631091

Amount

25 rxns

Lot Number

Specified on product label.

Product Information

The ProteoTuner Tag Kit provides you with a ProteoTuner tag sequence so you can quickly and easily convert any Living Colors® fluorescent protein-C vector into a ProteoTuner vector. The Kit contains a ProteoTuner destabilization domain (DD) source vector plus a primer mix that lets you PCR-amplify the DD tag sequence. You can then use an In-Fusion® Cloning Kit to insert the DD tag sequence upstream of the fluorescent protein coding sequence in any of our fluorescent protein-C vectors linearized at the AgeI site. The resulting vector will be able to express a protein of interest that is N-terminally-tagged with a fluorescent protein and subject to ligand-dependent degradation.

Package Contents

- 25 µl pDD Vector (20 ng/µl)
- 50 µl DD Primer Mix (10 µM)
Forward Primer: 5'-GCTAGCGCTACCGGTATGGGAGTGCAGGTGG-3'
Reverse Primer: 5'-ATGGTGGCGACCGGTCCTTCCGGTTTTAGAAGC-3'

Storage Conditions

- Store plasmids at -20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (-70°C)

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Product User Manuals

User manuals for Clontech products are available for download at www.clontech.com/manuals

The following user manual applies to this product:

- DD cDNA Amplification Protocol (PT5154-2)

pDD Vector Information

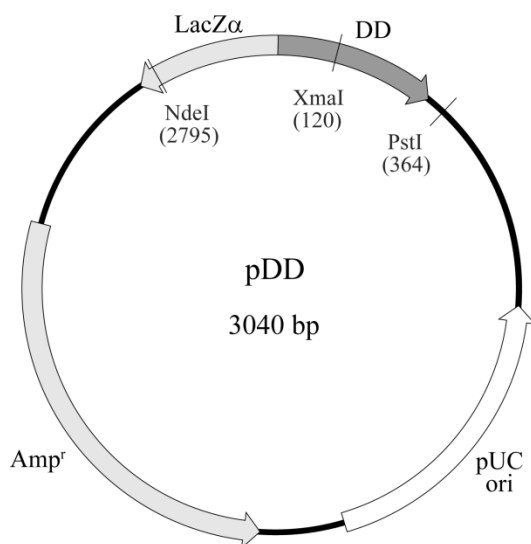


Figure 1. pDD Vector Map

Description

pDD is a pUC-based source vector encoding ProteoTuner destabilization domain (DD) cDNA, derived from FKBP (L106) [1, 2]. The vector was designed to provide DD tag coding sequence suitable for In-Fusion™ cloning into any Living Colors fluorescent protein-C vector. pDD contains a pUC origin of replication and an ampicillin resistance gene that allow propagation and selection in *E. coli*.

Location of Features

- DD (FKBP (L106P)-based ProteoTuner destabilization domain): 1–324
- pUC origin of replication: 791–1379 (complementary)
- Amp^r (Ampicillin resistance gene; β-lactamase): 1550–2410 (complementary)
- LacZα (N-terminal fragment of β-galactosidase): 2756–3040 (complementary)

Additional Information

The pDD vector is provided as part of the ProteoTuner Tag Kit. It is intended to be used as a template source for PCR amplification of DD cDNA.

Propagation in *E. coli*

- Recommended host strain: DH5α, XL1 Blue, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Yanisch-Perron, C., *et al.* (1985) *Gene* **33**(1):103-119.
2. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzymes	Fragment Sizes
XmaI	3.0 kb
NdeI & PstI	0.61 & 2.4 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280} : 1.8–2.0

Functional Test

- PCR was performed (*1 cycle*: 94°C for 15 sec; *25 cycles*: 98°C for 10 sec, 67°C for 25 sec) using the ProteoTuner Tag Kit components and Advantage® HD Polymerase Mix (Cat # 639241). When 10µl of the PCR product was run on a 1% agarose gel, a ~320 bp amplicon was observed.

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LICENSING STATEMENTS:

Patent pending.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.

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