Dnmt1 Chromobody\textsuperscript{®}-TagRFP plasmid

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by ChromoTek. This vector has not been completely sequenced.

For detailed sequence information, please contact info@chromotek.com

Location of features

- **PCMV IE**: 1-589
- Enhancer region: 59-465
- **TATA box**: 554-560
- Transcription start point: 583
- **Dmmt1-ViH**: 621-1004
- **TagRFP**: 1056-1769
- Stop codon: 1767-1769
- **SV40 early mRNA polyadenylation signal**: Polyadenylation signals: 1919-1924 & 1948-1953
- **mRNA 3' ends**: 1957 & 1969
- **t1 single-strand DNA origin**: 2016-2471
- **SV40 origin of replication**: 2812-2947
- **SV40 early promoter Enhancer (72-bp tandem repeats)**: 2648-2719 & 2720-2791
- **21-bp repeats**: 2795-2815, 2816-2836 & 2838-2858
- Early promoter element: 2871-2877
- **Kanamycin/neomycin resistance gene**: Neomycin phosphotransferase coding sequences: Start codon (ATG): 2999-3001; Stop codon: 3791-3793
- **Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal**: 4029-4034 & 4042-4047
- **ColE1 replication origin**: 4325-5007

**Product** | **Code** | **Size**
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pDC-TagRFP | dcr | 20 µg

**Vector type**: mammalian expression vector
**Reporter**: TagRFP
**Reporter codon usage**: mammalian
**Promoter for Chromobody\textsuperscript{®}**: **PCMV IE**
**Host cells**: mammalian
**Selection**: prokaryotic – kanamycin
**Replication**: prokaryotic
**Use**: Dnmt1 Chromobody\textsuperscript{®}-TagRFP expression in mammalian cells for non-invasive live cell visualization of endogenous nuclear Dmnt1.

**Vector description**

Dnmt1 Chromobody\textsuperscript{®}-TagRFP plasmid (pDC-TagRFP) is a mammalian expression vector encoding the epigenetic DNA (cytosine-5)-methyltransferase 1 marker Dnmt1-ViH fused to red fluorescent protein TagRFP (from Evrogen). The vector allows expression Dnmt1 Chromobody\textsuperscript{®}-TagRFP fusion protein in eukaryotic (mammalian) cells.

Chromobody\textsuperscript{®} codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

The vector backbone contains immediate early promoter of cytomegalovirus (PCMV \textsuperscript{IE}) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, ColE1 origin for replication of SV40 T-antigen, and HSV TK thymidine kinase (TK) polyadenylation signal for SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (PSV40) provides neomycin resistance gene (Neo\textsuperscript{5}) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P\textsuperscript{CMV}) provides kanamycin resistance gene expression (Kan\textsuperscript{r}) in E. coli. Kan\textsuperscript{r}/Neo\textsuperscript{5} gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

**Expression in mammalian cells**

pDC-TagRFP vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 [Gorman 1985].

**Propagation in E. coli**

Suitable host strains for propagation in E. coli include DH5\textsuperscript{alpha}, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to E. coli hosts. Copy number in E. coli is about 500.

Note: The plasmid DNA was isolated from dam\textsuperscript{-}methylated E.coli. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam\textsuperscript{-} host and make fresh DNA.

Notice to Purchaser:

Chromobody\textsuperscript{®}-related materials (the Products) are intended for research use only. The Products are covered by U.S. Pat. applications pending. By use of these Products, you accept the terms and conditions of the applicable End User License Agreement (EULA non-profit entities). The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

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