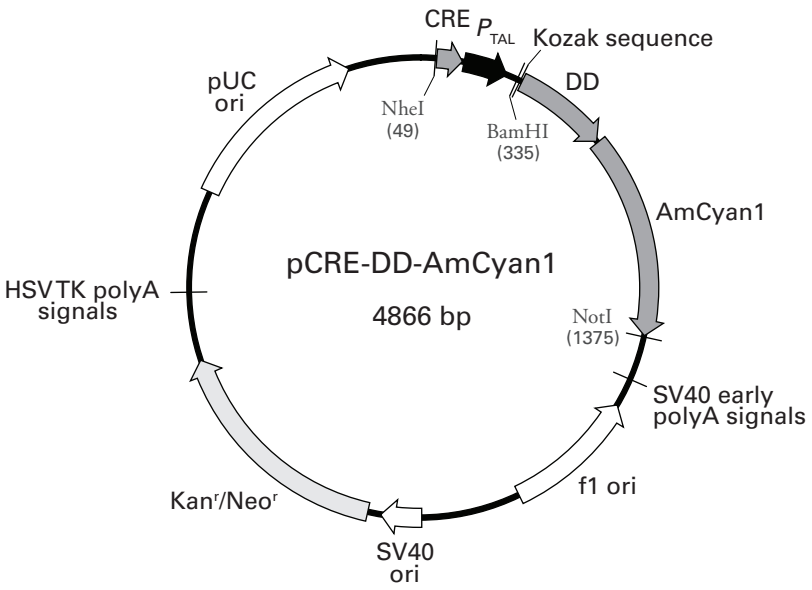
Vector Information

pCRE-DD-AmCyan1 Reporter Vector Information PT5121-5

Sold as part of Cat. No. 631089



pCRE-DD-AmCyan1 Reporter Vector Map

Description

pCRE-DD-AmCyan1 is a reporter vector that allows you to monitor cAMP response element binding protein (CREB) activation in mammalian cells. The vector contains two copies of the cAMP response element (CRE; 1) fused to a TATA-like promoter (P_{TAL}) region from the herpes simplex virus thymidine kinase (HSV-TK) gene. The vector encodes the reporter protein DD-AmCyan1, a ligand-dependent, destabilized cyan fluorescent protein that minimizes background fluorescence from leaky promoters.

AmCyan1 is a human codon-optimized variant of the wild-type *Anemonia majano* cyan fluorescent protein (AmCyan) that exhibits enhanced emission characteristics (excitation and emission maxima: 458 and 489, respectively; 2, 3). DD-AmCyan1 is a modified version of AmCyan1 that is tagged on its N-terminus with the ProteoTuner™ destabilization domain (DD; 4). The presence of this destabilization domain causes rapid, proteasomal degradation of the fluorescent fusion protein; however, when the membrane permeant ligand Shield1 is added to the medium, it binds to the destabilization domain and protects the fusion protein from degradation.

In the absence of Shield1, the destabilization domain causes the degradation of any DD-AmCyan1 reporter protein produced prior to promoter activation, thus reducing background fluorescence. In order to analyze CREB activation, an inducer of choice is added to the medium along with the Shield1 stabilizing ligand, which effectively stabilizes the reporter protein, allowing it to accumulate. As a result, only the reporter molecules expressed during promoter induction will contribute to the fluorescence signal, providing a considerably higher signal-to-noise ratio than that obtained with non-destabilized or constitutively destabilized reporter systems. The high signal-to-noise ratio also allows the monitoring of CREB activation during discrete windows of time when Shield1 is added to the cell medium for discrete periods of time.

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The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (5).This cassette consists of the SV40 early promoter, aTn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSVTK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

The pCRE-DD-AmCyan1 Reporter vector, available as part of the CRE DD Cyan Reporter System (Cat. No. 631089), can be used to monitor CREB activation in live cells as well as *in vivo*. pCRE-DD-AmCyan1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418.

Location of features

- CRE (cAMP response element): 54–140
- P_{TAL} (TATA-like promoter): 147–295
- Kozak sequence: 346–356
- DD-AmCyan1
  - Start codon (ATG): 353–355; Stop codon: 1370–1372
  - DD (destabilization domain; 3): 353–676
  - AmCyan1 (*Anemonia majano* cyan fluorescent protein): 683–1369
- SV40 early polyA signals: 1525–1559
- f1 origin of replication: 1622–2077 (complementary)
- SV40 origin of replication: 2418–2556
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  - Neomycin phosphotransferase coding sequences: 2602–3396
- HSVTK polyA signals: 3632–3650
- pUC origin of replication: 3981–4624

Propagation in *E. coli*

- Recommended host strains: DH5α™, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 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
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of AmCyan1

- Excitation maximum = 458 nm
- Emission maximum = 489 nm

References

1. Himmler, A. *et al.* (1993) *J. Recep. Res.* **13**(1–4):79–94.
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3. Haas, J. *et al.* (1996) *Curr. Biol.* **6**(3):315-324
4. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech.This vector has not been completely sequenced.

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The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.