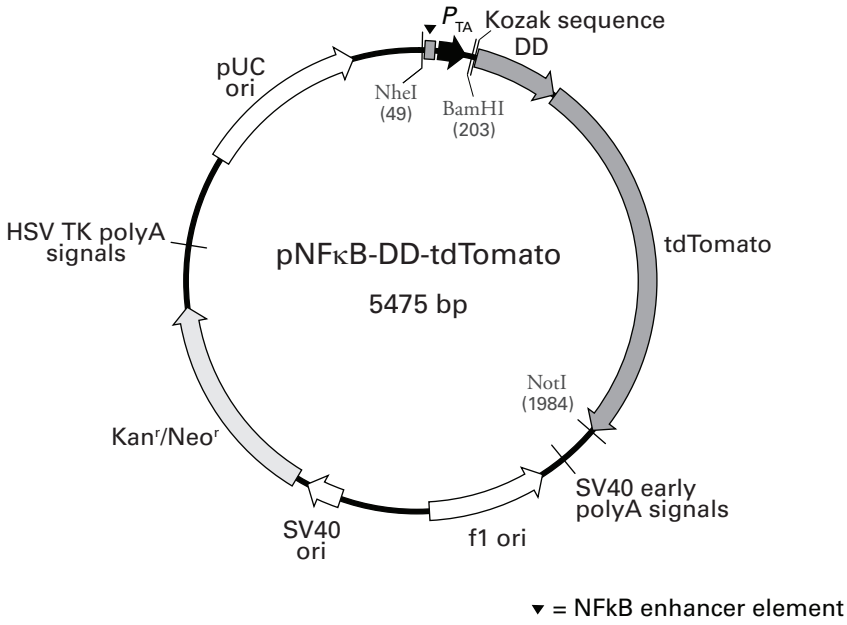
Vector Information

pNFκB-DD-tdTomato Reporter Vector Information PT5117-5

Sold as part of Cat. No. 631081



pNFkB-DD-tdTomato Reporter Vector Map

Description

pNFκB-DD-tdTomato is a reporter vector that allows you to monitor NFκB activation in mammalian cells. The vector contains an NFκB enhancer element (composed of four tandem copies of the NFκB consensus binding sequence; 1) upstream of a minimal TA promoter (P_{TA}), which consists of the TATA box from the herpes simplex virus thymidine kinase (HSV-TK) promoter. The vector encodes the reporter protein DD-tdTomato, a ligand-dependent, destabilized red fluorescent protein that minimizes background fluorescence from leaky promoters.

tdTomato is a member of the family of fruit fluorescent proteins derived from the *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 554 and 581 nm, respectively; 1, 2). DD-tdTomato is a modified version of tdTomato that is tagged on its N-terminus with the ProteoTuner™ destabilization domain (DD; 3). 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-tdTomato reporter protein produced prior to promoter activation, thus reducing background fluorescence. In order to analyze NFκB 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 NFκB 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 (4). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

The pNFκB-DD-tdTomato Reporter vector, available as part of the NFκB DD Red Reporter System (Cat. No. 631081), can be used to monitor NFκB activation in live cells as well as *in vivo*. pNFκB-DD-tdTomato can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418.

Location of features

- NFκB enhancer element: 54–93
- P_{TA} (TA minimal promoter): 113–119
- Kozak sequence: 214–224
- DD-tdTomato
  - Start codon (ATG): 221–223; Stop codon: 1979–1981
  - DD (FKBP-L106P destabilization domain; 3): 221–544
  - tdTomato: 551–1978
- SV40 early polyA signals: 2134–2168
- f1 origin of replication: 2231–2686 (complementary)
- SV40 origin of replication: 3027–3165
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  - Neomycin phosphotransferase coding sequences: 3211–4005
- HSV TK polyA signals: 4241–4259
- pUC origin of replication: 4590–5233

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 tdTomato

- Excitation maximum = 554 nm
- Emission maximum = 581 nm

References

1. Pessara, U. & Koch, N. (1990) *Mol. Cell Biol.* **10**(8):4146–4154.
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3. Campbell, R. E. et al. (2002) *Proc. Natl. Acad. Sci. USA* **99**(12):7877–7882.
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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