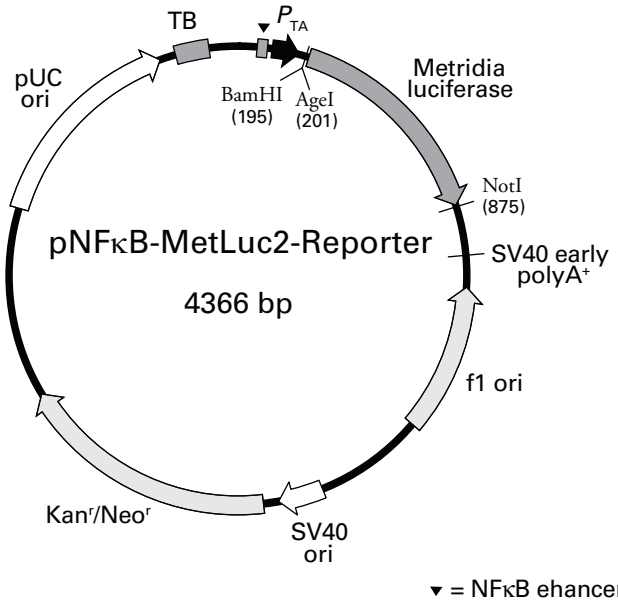
Vector Information

pNFκB-MetLuc2-Reporter Vector Information

PT4071-5

Catalog No. 631743



pNFκB-MetLuc2-Reporter Vector Map. All restriction sites shown are unique.

Description:

pNFκB-MetLuc2-Reporter allows you to monitor the activation of the NFκB signal transduction pathway (1–4) directly from the cell culture medium, without cell lysis. The vector contains an NFκB enhancer element (composed of four tandem copies of the NFκB consensus binding sequence; 5), located upstream of the minimal TA promoter (P_{TA}), which consists of the TATA box from the herpes simplex virus thymidine kinase (HSV-TK) promoter. Located downstream of P_{TA} is a human codon optimized secreted-luciferase gene from the marine copepod *Metridia longa*. This gene encodes a 219 amino acid (24 kDa) polypeptide that includes a 17 amino acid N-terminal signal peptide necessary for secretion. Binding of transcription factors to the NFκB enhancer element allows *Metridia* luciferase (MetLuc) to be expressed and secreted into the surrounding medium (6).

To prevent read-through transcription of the *Metridia* luciferase gene, a synthetic transcription blocker (TB; 7, 8), composed of adjacent polyadenylation and transcription pause sites, is located upstream of the *cis*-acting enhancer element. To ensure efficient processing of the luciferase transcript in eukaryotic cells, an SV40 early polyadenylation signal is located downstream of the *Metridia* luciferase coding sequence. The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and a kanamycin resistance gene for propagation and selection in *E. coli*.

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Use:
pNFκB-MetLuc2-Reporter is available as part of the Ready-To-Glow™ NFκB Secreted Luciferase Reporter System (Cat. No. 631743). In mammalian cells containing pNFκB-MetLuc2-Reporter, the addition of TNFα, IL-1, or stimulants of lymphokine receptors to the culture medium induces transcription factors to bind to the NFκB enhancer element, thereby initiating transcription of the secreted luciferase reporter gene. Activation of the NFκB signal transduction pathway can be monitored simply by sampling the culture medium. To monitor the effect of a specific protein on the pathway, pNFκB-MetLuc2-Reporter can be cotransfected with an expression vector containing the gene of interest. pNFκB-MetLuc2-Reporter can be transfected into mammalian cells using any standard transfection method. Stable transfectants can be selected using G418 when required.

Location of features:
- NFκB enhancer element (5): 46–85
- P_{TA} (TA minimal promoter): 105–111
- *Metridia longa* secreted luciferase (human codon optimized):
  start codon (ATG): 213–215; stop codon: 869–871
- SV40 early polyadenylation signal: 1025–1030
  mRNA 3' end: 1063
- f1 origin of replication: 1122–1577
- SV40 origin of replication: 1918–2056
- Kan^r/Neo^r (kanamycin/neomycin resistance gene):
  Neomycin phosphotransferase coding sequence
  Start codon (ATG): 2102–2104; stop codon: 2894–2896
- pUC origin of replication: 3481–4124
- TB (transcription blocker; 7, 8): 4154–4307

Propagation in *E. coli*:
- Suitable host strains: DH5α™, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F' episome such as JM109 and XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Plasmid incompatibility group: pMB1/Col E1

References:
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