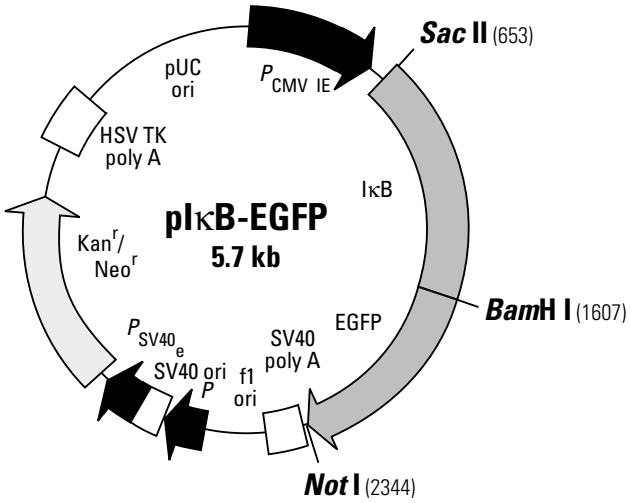

Restriction Map of pIκB-EGFP. All sites shown are unique.

Description:

The pIκB-EGFP Vector encodes the IκB-EGFP Signaling Probe, which is a fusion of enhanced green fluorescent protein (EGFP) and IκB. IκB is an inhibitor of NFκB, a transcription factor involved in the immune response and inflammatory diseases. When the NFκB pathway is inactive, IκB and NFκB exist as an inactive complex in the cytosol. Upon stimulation, IκB is degraded. In cells transfected with pIκB-EGFP, degradation of the IκB-EGFP fusion protein is observed as a decrease in EGFP fluorescence (1). The IκB-EGFP Signaling Probe is constitutively expressed and resides in the cytosol.

EGFP is a red-shifted, human codon-optimized variant of GFP (2–6) that has been engineered for brighter fluorescence and higher expression in mammalian cells. Its excitation maximum is 488 nm and emission maximum is 509 nm. For more information on the properties of EGFP, please refer to the BD Living Colors™ User Manual (PT2040-1) included with the vector.

Expression of the IκB-EGFP is driven by the human CMV immediate-early promoter. The SV40 poly-A sequence directs proper processing of the 3' end of the fusion construct. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r) allows kanamycin selection in *E. coli* and neomycin (G418) selection in eukaryotic cells. This cassette consists of the SV40 early promoter, the neomycin/ kanamycin resistance gene of Tn5, and poly-A signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

The IκB-EGFP Signaling Probe can be used to monitor activation of the NFκB signalling pathway by observing changes in EGFP fluorescence. EGFP can be detected by fluorescence microscopy or by flow cytometry. No antibody staining or fixation is necessary.

This vector is not intended for use as a cloning vector; however, there are sites flanking the EGFP and IκB coding regions for subcloning these sequences into other expression vectors. The vector can be transfected into mammalian cells using any standard method. If required, stable transformants can be selected using G418 (7).

(PR29012; published 04 October 2002)

Location of Features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- IκB gene: 655–1605
- Enhanced green fluorescent protein gene: 1621–2337
  Start codon (ATG): 1621–1623
  Insertion of Val at position 2: 1624–1626
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 1813–1818
  His-231 to Leu mutation (A→T): 2315
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2494–2499 & 2523–2528; mRNA 3' ends: 2532 & 2544
- f1 single-strand DNA origin: 2591–3046 (Packages the noncoding strand of IκB-EGFP)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 3108–3113; –10 region: 3131–3136
  Transcription start point: 3143
- SV40 origin of replication: 3387–3522
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 3220–3291 & 3292–3363
  21-bp repeats: 3367–3387, 3388–3408 & 3410–3430
  Early promoter element: 3443–3449
  Major transcription start points: 3439, 3477, 3483 & 3488
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3571–3573; stop codon: 4363–4365
  G→A mutation to remove *Pst* I site: 3753
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4099
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4601–4606 & 4614–4619
- pUC plasmid replication origin: 4950–5593

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 1687–1666
- EGFP-C Sequencing Primer (#6478-1): 2274–2295

- Suitable 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) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Mercury EGFP Signaling Probes (July 1999) *CLONTECHniques* **XIV**(3):22–24.
2. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
3. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
4. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
5. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

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