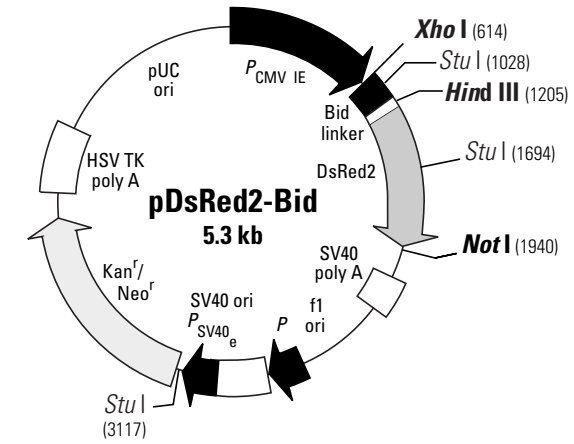
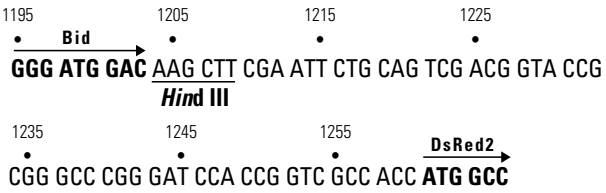
Vector Information

pDsRed2-Bid Vector Information PT3637-5

Cat. No. 632419





Restriction map and linker sequence of pDsRed2-Bid. Unique restriction sites are in bold. The *Not* I site follows the DsRed2 stop codon.

Description

pDsRed2-Bid is a mammalian expression vector that encodes a fusion of *Discosoma sp.* red fluorescent protein (DsRed2; 1, 2) and Bid, a member of the Bcl-2 "pro-apoptosis" family (3). Developed for our ApoAlert® line, pDsRed2-Bid is designed to help researchers study Bid-dependent apoptosis pathways. Because of its fluorescent label, the Bid-DsRed2 fusion is easily detected by microscopy, allowing researchers to track its movements in response to certain apoptosis inducing agents (e.g., TNF-α, Cat No. 630203).

Bid's activity is closely tied to its location in the cell. In healthy, non-apoptotic cells, Bid normally resides in the cytosol. But soon after the induction of apoptosis, it translocates to mitochondria, where it stimulates the release of cytochrome c—a key amplification step in the apoptotic cascade (4–7). The translocation is triggered by caspase-8, which, when activated by a death signal, cleaves Bid to produce a 15-kDa C-terminal fragment. The fragment, often referred to as truncated Bid or tBid, transmits the death signal further by translocating to the mitochondria.

You can monitor this event visually by expressing Bid as a DsRed2 fusion. Bid-DsRed2, the fusion expressed by this vector, for example, emits red fluorescence—even after truncation—so it can be followed as it moves from the cytosol to the mitochondria. To drive expression of Bid-DsRed2, this vector contains the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$), positioned just upstream of the Bid sequence. A short linker joins the Bid coding sequence to the 5'-end of DsRed2. Farther downstream, the vector contains a pair of SV40 polyadenylation signals, which direct proper processing of the 3'-end of the Bid-DsRed2 mRNA. The vector also contains an SV40 origin for replication in mammalian cells expressing the SV40 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), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418 (8). A bacterial promoter upstream of the cassette confers kanamycin resistance (Kan^r) to *E. coli*.

Use

Cells transfected with pDsRed2-Bid constitutively express Bid as an N-terminal fusion to DsRed2. pDsRed2-Bid can be introduced into a variety of mammalian cell lines using any standard transfection method. If required, stable transfectants can be selected using G418 (8).

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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
- Bid-DsRed2 fusion gene: 619–1938
  Human Bid gene: 619–1203
  Linker sequence: 1204–1260
  Discosoma sp. Red Fluorescent Protein (DsRed2) gene (human codon-optimized; 9): 1261–1938
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2090–2095 & 2119–2124; mRNA 3' ends: 2128 & 2140
- f1 single-strand DNA origin: 2187–2642 (Packages the noncoding strand of DsRed2.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2704–2709; –10 region: 2727–2732
  Transcription start point: 2739
- SV40 origin of replication: 2983–3118
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2816–2887 & 2888–2959
  21-bp repeats: 2963–2983, 2984–3004 & 3006–3026
  Early promoter element: 3039–3045
  Major transcription start points: 3035, 3073, 3079 & 3084
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 3167–3169; stop codon: 3959–3961
  G→A mutation to remove *Pst* I site: 3349
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3695
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4197–4202 & 4210–4215
- pUC plasmid replication origin: 4546–5189

Propagation in *E. coli*

- 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

Red Fluorescent Protein (DsRed2)

- Excitation/Emission Maxima: 558 nm / 583 nm

References

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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 Clontech. This vector has not been completely sequenced.