

USER GUIDE

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by *life* technologies™

pSecTag2 A, B, and C

Catalog number V900-20

Revision date 19 January 2012

Publication Part number 28-0159

MAN0000651

For Research Use Only. Not for diagnostic procedures.

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Kit Contents and Storage

Shipping and Storage

pSecTag2 vectors are shipped on wet ice. Upon receipt, store vectors at -20°C .

Kit Contents

All vectors are supplied as detailed below. **Store the vectors at -20°C .**

Item	Composition	Amount
pSecTag2 A, B, and C	40 μL of 0.5 $\mu\text{g}/\mu\text{L}$ vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 μg
pSecTag2/PSA	40 μL of 0.5 $\mu\text{g}/\mu\text{L}$ vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 μg

Intended Use

For research use only. Not intended for any human or animal diagnostic or therapeutic uses.

Introduction

Product Overview

Description of the System

pSecTag2 A, B, and C are 5.2 kb expression vectors designed for high-level stable and transient expression in mammalian hosts. The pSecTag2 vectors represent an improvement over the pSecTag vectors. Potential stop codons have been removed from the multiple cloning sites to facilitate cloning in frame with the N-terminal and/or the C-terminal peptide sequence.

Proteins expressed from pSecTag2 are fused at the N-terminus to the murine Ig κ chain leader sequence for protein secretion and at the C-terminus to a peptide containing the *c-myc* epitope and six tandem histidine residues for detection and purification. The Zeocin™ resistance gene allows selection in both *E. coli* and mammalian cells in the presence of the antibiotic Zeocin™ (for general and purchasing information on Zeocin™, refer to page 12). The pSecTag2 vector is supplied in three different versions (A, B, and C) to facilitate correct in-frame fusion with the Ig κ -chain leader sequence.

To get started with cloning into pSecTag2, see page 2.

For more information on the pSecTag2 vector, see page 9.

Experimental Outline

Use the following outline to clone and express your gene of interest in pSecTag2:

1. Consult the multiple cloning sites described on pages 3-5 to determine which vector (A, B, or C) should be used to clone your gene in frame with the C-terminal *myc* epitope and the polyhistidine tag.
 2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50–100 $\mu\text{g}/\text{mL}$ ampicillin or 25–50 $\mu\text{g}/\text{mL}$ Zeocin™ in Low Salt LB. For more information, see page 13.
 3. Analyze your transformants for the presence of insert by restriction digestion.
 4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in-frame with the C-terminal peptide.
 5. Transfect your construct into the cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
 6. Test for expression of your recombinant gene by western blot analysis or functional assay. For antibodies to the *myc* epitope or the C-terminal polyhistidine tag, see page 14.
 7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 14 for ordering information).
-

Methods

Cloning into pSecTag2

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, see *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989), or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector including TOP10F and DH10B™. We recommend propagating vectors containing inserts in *E. coli* strains that are recombination deficient (*recA*) and endonuclease A deficient (*endA*)

For your convenience, TOP10F' is available from Life Technologies as chemically competent or electrocompetent cells (see page 14).

Note that any *E. coli* strain that contains the complete Tn5 transposable element (i.e. DH5αF'IQ, SURE, SURE2) encodes the *ble* (bleomycin) resistance gene. These strains will be resistant to Zeocin™. For the most efficient selection it is highly recommended that you choose an *E. coli* strain that does not contain the Tn5 gene.

Maintaining pSecTag2

To propagate and maintain pSecTag2 A, B, and C, we recommend that you transform the plasmids into *E. coli* and prepare glycerol stocks for long-term storage.

To transform plasmids into *E. coli*, use a small amount of the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a *recA*, *endA* *E. coli* strain like TOP10F, DH10B™ or equivalent.

Note: Do not use strains that contain the Tn5 transposon (i.e., DH5 F IQ, SURE, SURE2). These strains contain the bleomycin resistance gene (*ble*) that confers resistance to Zeocin™.

Transformants are selected on LB plates containing 50–100 µg/mL ampicillin or on Low Salt LB plates containing 25 µg/mL Zeocin™ (see page 13 for the recipe for Low Salt LB medium containing Zeocin™, or page 14 for ordering information).

Analyze transformants for the appropriate plasmid and prepare glycerol stocks by mixing 0.85 mL of an overnight culture with 0.15 mL of sterile glycerol. Transfer the resulting solution to a cryovial and store at –80°C.

Cloning into the pSecTag2 Vectors

pSecTag2 A, B, and C vectors are fusion vectors requiring that you clone your gene of interest in frame with the initiation ATG of the N-terminal I κ g-chain leader sequence and/or the C-terminal *myc* epitope/polyhistidine tag. Three versions of this vector are provided to facilitate cloning. For proper expression, first determine which restriction sites are appropriate for ligation and then which vector will preserve the reading frame at **BOTH** the 5' and the 3' ends. It may be necessary to PCR your gene product to create a fragment with the appropriate restriction sites to clone in frame at both ends. Carefully inspect your gene and the multiple cloning site of each vector before cloning your gene of interest. See pages 3–5 for details of the multiple cloning sites.

Continued on next page

Cloning into pSecTag2, continued

Note

If you wish to express and secrete your protein without the C-terminal tag, include a termination codon in your gene of interest. Note that you will be unable to detect the fusion protein with the Anti-*myc* Antibody or Anti-His (C-term) Antibody or purify it using nickel-chelating resin (i.e., ProBond™).

Multiple Cloning Site of pSecTag2 A

Below is the multiple cloning site for pSecTag2 A. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the Igκ-chain leader sequence. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pSecTag2 A is available for downloading from our website (www.lifetechnologies.com) or from **Technical Support**(see page 15).

```

699      enhancer region (3' end)
      AATGGGAGTT TGTTTTGGCA CAAAATCAA CGGGACTTTC CAAAATGTCG TAACAACCTCC

759      CAAT
      GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT AAGCAGAGCT
      putative transcriptional start
      CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT

819      T7 promoter primer binding site
      CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT

879      Ig κ-chain leader sequence
      AGGGAGACCC AAGCTGGCTA GCCACC ATG GAG ACA GAC ACA CTC CTG CTA TGG
      Met Glu Thr Asp Thr Leu Leu Leu Trp
      Sfi I
932      GTA CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC GCG GCC CAGCCG
      Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp
      Signal cleavage site
980      Asc I      Hind III      Asp718 I      Kpn I      BamH I      BstX I*      EcoR I
      GCCAGGCGC [GCCG] TACGAAG CTTGGTACCG AGCTCGGATC CACTCCAGTG TGGTGGAAATT

1040      Pst I      EcoR V      BstX I*      Not I      Xho I      Dra II      Apa I
      CTGCAGATAT CCAGCACAGT GCGGCGCCGCT CGAGGAGGGC CC GAA CAA AAA CTC ATC
      Glu Gln Lys Leu Ile
      Polyhistidine tag
1097      TCA GAA GAG GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT CAT TGA
      Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His His His His His His ***

1148      BGH reverse priming site
      GTTTAAACCC GCTGATCAGC CTCGACTGTG CTTCTAGTT GCCAGCCATC TGTTGTTTGC
      BGH poly (A) addition site
1208      CCCTCCCCCG TGCCTTCCTT GACCCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA

1268      AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGT
  
```

*Note that there are two *BstX I* sites in the polylinker.

Continued on next page

Cloning into pSecTag2, continued

Multiple Cloning Site of pSecTag2 B

Below is the multiple cloning site for pSecTag2 B. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the Ig κ -chain leader sequence. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pSecTag2 B is available for downloading from our website (www.lifetechnologies.com) or from **Technical Support** (see page 15).

```

        enhancer region (3' end)
199  AATGGGAGTT TGTTTTGGCA CAAAATCAA CGGGACTTTC CAAAATGTCG TAACAACTCC

        CAAT
759  GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCCTATAT AAGCAGAGCT
        putative transcriptional start
        T7 promoter primer binding site
819  CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT

        Ig  $\kappa$ -chain leader sequence
879  AGGGAGACCC AAGCTGGCTA GCCACC ATG GAG ACA GAC ACA CTC CTG CTA TGG
        Met Glu Thr Asp Thr Leu Leu Leu Trp
        Sfi I
932  GTA CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC GCG GCC CAGCCG
        Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp
        Signal cleavage site
        Hind III Asp718 I Kpn I BamHI BstX I* EcoR I
980  GCCAGGCGC GCGCGCCG TACGAAG CTTGGTACCG AGCTCGGATC CACTCCAGTG TGGTGGA

        Pst I EcoR V BstX I* Not I Xho I Dra II Apa I
1041 ATTCTGCAGA TATCCAGCAC AGTGGCGGCC GCTCGAGGAG GGCCC GAA CAA AAA CTC
        Glu Gln Lys Leu

        myc epitope
1098 ATC TCA GAA GAG GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT
        Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His His His His His

        BGH reverse priming site
1146 CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CTTCTAGTT GCCAGCCATC
        His ***

1202 TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT

        BGH poly (A) addition site
1262 TTCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGT
    
```

*Note that there are two *BstX I* sites in the polylinker.

Continued on next page

Cloning into pSecTag2, continued

Multiple Cloning Site of pSecTag2 C

Below is the multiple cloning site for pSecTag2 C. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the Ig κ -chain leader sequence. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pSecTag2 C is available for downloading from our website (www.lifetechnologies.com) or from **Technical Support** (see page 15).

```

699      enhancer region (3' end)
      AATGGGAGTT TGT TTTGGCA CAAAATCAA CGGGACTTTC CAAAATGTCG TAACAACTCC

759      CAAT                                TATA                                3' end of hCMV
      GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCCTATAT AAGCAGAGCT
      putative transcriptional start                                T7 promoter primer binding site

819      CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT

879      Ig  $\kappa$ -chain leader sequence
      AGGGAGACCC AAGCTGGCTA GCCACC ATG GAG ACA GAC ACA CTC CTG CTA TGG
      Met Glu Thr Asp Thr Leu Leu Leu Trp

932      Sfi I
      GTA CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC GCG GCC CAGCCG
      Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp
      Signal cleavage site

980      Hind III Asp718 I Kpn I BamH I
      GCCAGGCGC GCGCGCCGTACG TACGAAG CTTGGTACCG AGCTCGGATC CACTCCAGTG

1038      BstX I* EcoR I Pst I EcoR V BstX I* Not I Xho I Dra II Apa I
      TGGTGGAAAT CTGCAGATAT CCAGCACAGT GCGGCGCCGCT CGAGGAGGGC CC GAA CAA
      Glu Gln

1096      myc epitope                                Polyhistidine tag
      AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT
      Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His His His

1144      BGH reverse priming site
      CAT CAT CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CTTCTAGTT
      His His His ***

1196      GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC

1256      BGH poly (A) addition site
      CCACTGTCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGT
  
```

*Note that there are two *BstX* I sites in the polylinker.

Transformation and Transfection

Introduction

At this point you should have ligation mixtures that are ready to be transformed into competent *E. coli*. The following guidelines and recommendations are provided for your convenience. If you need more details about the techniques discussed, refer to the general molecular biology references (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

E. coli Transformation

Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10F', DH10B™) and select on LB plates containing 50–100 µg/mL ampicillin or Low Salt LB plates containing 25 µg/mL Zeocin™ (see page 13). Select 10–20 clones and analyze for the presence and orientation of your insert.



We recommend that you sequence your construct with the T7 forward and BGH reverse primers to confirm that your gene is correctly fused to the Igκ-chain leader sequence at the N-terminal and the C-terminal tag. For ordering primers, see page 14.

Plasmid Preparation

Once you have confirmed that your gene is in the correct reading frame, prepare plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink® HiPure Miniprep Kit or the PureLink® HiPure Midiprep Kit (see page 14 for ordering information).

Methods of Transfection

For established cell lines (e.g. HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). Life Technologies offers the Lipofectamine® 2000 Reagent for mammalian transfection (page 14). For more information, see our website (www.lifetechnologies.com) or contact **Technical Support** (page 15).

Creating Stable Cell Lines

Handling Zeocin™

- High salt and acidity or basicity inactivates Zeocin™. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see page 13).
Note: The salt concentration should not be adjusted for mammalian cells. Changes to the salt concentration are detrimental to cells.
 - Store Zeocin™ at -20°C and thaw on ice before use.
 - Zeocin™ is light sensitive. Store drug, plates, and medium containing drug in the dark.
 - Wear gloves, a laboratory coat, and safety glasses or goggles when handling solutions containing Zeocin™.
 - Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.
-

Determining Zeocin™ Sensitivity

To obtain a stable integrant, you must first determine if the cell line in question can grow as an isolated colony. You may already know this for your cell line. If you do not, seed ~100 cells in a 60 mm plate and feed every 4 days for 10–12 days. Count the number of colonies. Growing in soft agar can help cells to grow when they are diluted; however, some cell lines (e.g. NIH3T3) require plating at a certain density in order to grow properly (Ausubel *et al.*, 1994).

Next, determine the minimal concentration of Zeocin™ required to prevent growth of the parental cell line using the protocol below:

1. Plate or split a confluent plate so there are approximately 2.5×10^5 cells per 60–100 mm dish. Prepare 7 plates and add varying concentrations of Zeocin™ (0, 50, 125, 250, 500, 750, and 1000 µg/mL) to each plate.
 2. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.
 3. Count the number of viable cells at regular intervals to determine the appropriate concentration of Zeocin™ that prevents growth.
-

Selecting Stable Integrants

Once you have determined the appropriate Zeocin™ concentration to use, you can generate a stable cell line with your construct.

1. Transfect cells with your construct using the desired protocol and plate. Remember to include a plate of untransformed cells as a negative control.
 2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
 3. 48 hours after transfection, split the cells into fresh medium containing Zeocin™ at the pre-determined concentration required for your cell line. Split the cells such that the cells are no more than 25% confluent.
 4. Feed the cells with selective medium every 3–4 days until foci can be identified.
 5. Pick and expand 20–40 foci to test for expression of your recombinant protein.
-

Expression and Purification

Introduction

Expression of your recombinant protein can be detected using an antibody to the *c-myc* epitope encoded in the C-terminal fusion peptide. In addition, the metal binding domain allows simple, one-step purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using a nickel-chelating resin.(i.e., ProBond™, see page 14 for ordering).

Detecting Secreted Protein from Medium

The medium in which transfected cells are grown can be analyzed for secreted, recombinant protein by functional assay or western blot analysis. It may be necessary to perform a time course analysis to determine the optimal time for expression of your recombinant protein. If you do not have antibody to your particular protein, you can use the Anti-*myc* or Anti-His(C-term) antibodies to detect your protein.

A positive control vector, pSecTag2/PSA, is included to test for expression and secretion in your particular cell line. Prostate-specific antigen (PSA) is fused to the *c-myc* epitope and the polyhistidine tag. The resulting fusion protein is ~33 kDa which includes the N-terminal secretion signal. The signal adds 2.4 kDa to the fusion protein. **Note:** There are glycosylation sites in PSA. The secreted fusion protein expressed in human breast carcinoma cells migrates at ~45 kDa. The PSA protein is processed via the normal secretory pathway, but is glycosylated during this process.

Purifying Secreted Recombinant Protein

To purify secreted, recombinant protein from the medium, follow the manufacturer's instructions for the nickel-chelating resin that you are using. Start with about 3–5 mL of medium and load onto 1–2 mL of resin. Scale up or down depending on the level of expression.

Analyzing Cells for Recombinant Protein

If you do not detect any secreted protein in the medium, use the procedure below to check cells for production of recombinant protein. You will need 5×10^6 to 1×10^7 cells for purification on a 2 mL ProBond™ column (see ProBond™ Purification System manual).

1. Seed cells in five T-75 flasks or two to three T-175 flasks.
 2. Grow the cells in selective medium until they are 80–90% confluent.
 3. Harvest the cells by treating with trypsin-EDTA for 2–5 minutes or by scraping the cells in PBS.
 4. Inactivate the trypsin by diluting with medium and transfer the cells to a sterile conical tube.
 5. Centrifuge the cells at $240 \times g$ for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at -80°C until needed.
-

Lysis of Cells

If you are using ProBond™ resin, refer to the ProBond™ Purification System manual for details about sample preparation for chromatography.

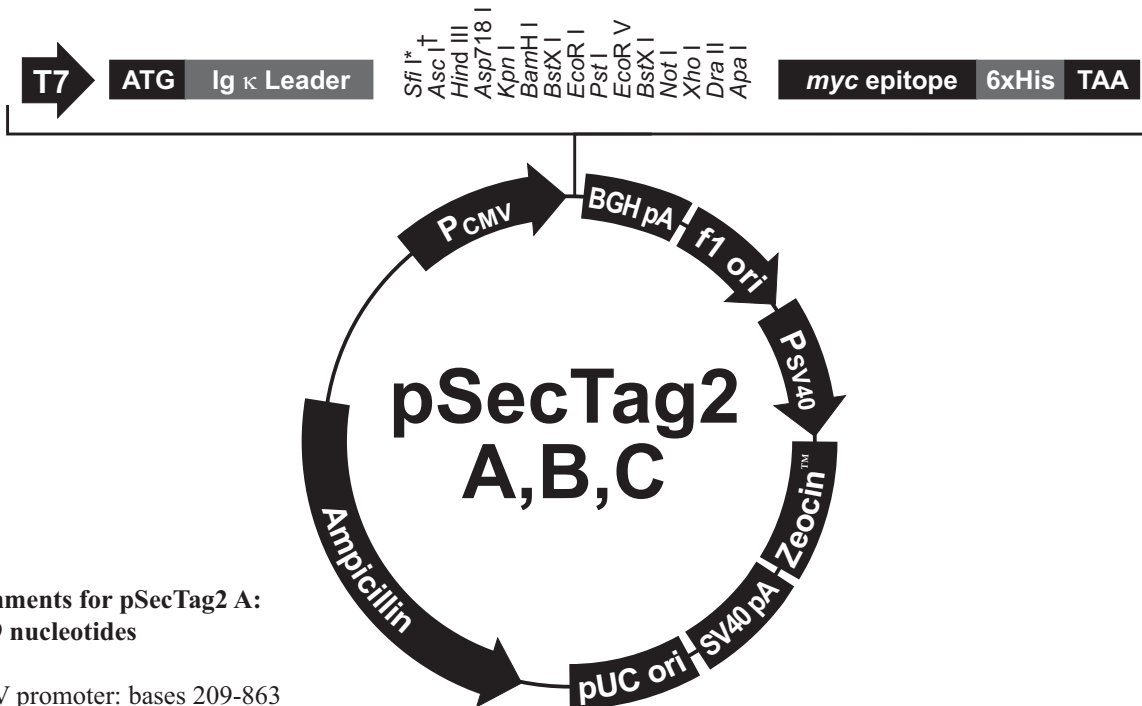
If you are using other resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

pSecTag2 Vector

Map of pSecTag2

The figure below shows where the features of pSecTag2 are located in the vector. The sequences for pSecTag2 A, B, and C are available for downloading from our website (www.lifetechnologies.com) or from **Technical Support** (see page 15). Details of the multiple cloning sites for all three vectors, including the variable region that determines the reading frame, are shown on pages 3-5.



Comments for pSecTag2 A: 5159 nucleotides

- CMV promoter: bases 209-863
- T7 promoter/priming site: bases 863-882
- Murine Ig κ-chain V-J2-C signal peptide: bases 905-967
- Multiple cloning site: bases 970-1081
- c-myc* epitope: bases 1082-1114
- Polyhistidine tag: bases 1127-1144
- BGH reverse priming site: bases 1167-1184
- BGH polyadenylation sequence: bases 1166-1380
- f1 origin: bases 1443-1856
- SV40 promoter and origin: bases 1924-2245
- EM-7 promoter: bases 2261-2327
- Zeocin™ resistance gene: bases 2328-2702
- SV40 polyadenylation sequence: bases 2832-2961
- pUC origin: bases 3345-4018
- Ampicillin resistance gene: bases 4163-5023 (C)

* The *Sfi* I site is located before the variable region.

†Frame-dependent variations:
Asc I is only found in version A.

Continued on next page

pSecTag2 Vector, continued

Features of pSecTag2

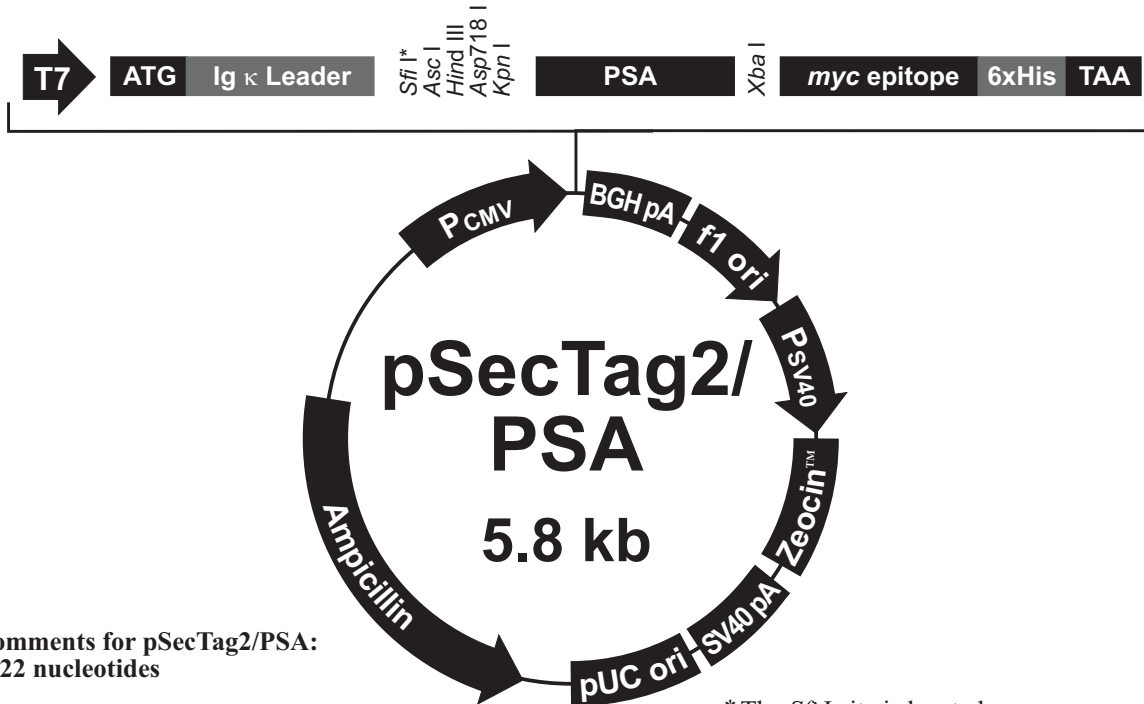
pSecTag2 A (5159 bp), pSecTag2 B (5163 bp), and pSecTag2 C (5167 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987).
T7 promoter/priming site	Allows for <i>in vitro</i> transcription in the sense orientation and sequencing through the insert.
ATG initiation codon	Permits initiation of translation of the pSecTag2 fusion protein.
Murine Ig κ -chain leader sequence	Allows secretion of the fusion protein (Coloma <i>et al.</i> , 1992).
Multiple cloning site	Allows insertion of your gene and facilitates cloning.
<i>c-myc</i> epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of pSecTag2 fusion protein with the Anti- <i>myc</i> antibodies (Evan <i>et al.</i> , 1985) (see page 14 for ordering).
Polyhistidine tag	For high affinity binding to Ni ²⁺ -chelating resin (i.e. ProBond™) and easy purification. In addition, it allows detection of pSecTag2 fusion proteins with the Anti-His(C-term) antibodies (Lindner <i>et al.</i> , 1997) (see page 14 for ordering).
BGH reverse priming site	Permits sequencing through the insert.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992).
f1 origin	Allows rescue of single-strand DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the Zeocin™ resistance gene and episomal replication in cells expressing the SV40 large T antigen.
Zeocin™ resistance gene (<i>Sh ble</i>)	For selection of transformants in <i>E. coli</i> and stable transfectants in mammalian cells (Drocourt, <i>et al.</i> , 1990; Mulsant, <i>et al.</i> , 1988).
SV40 polyadenylation signal	Permits efficient transcription termination and polyadenylation of mRNA.
pUC origin	Allows high-copy number replication and growth in <i>E. coli</i> .
Ampicillin resistance gene (β -lactamase)	Permits selection in <i>E. coli</i> .

pSecTag2/PSA

Map of pSecTag2/PSA

The figure below shows the features of pSecTag2/PSA. The vector sequence for pSecTag2/PSA is available for downloading from our website (www.lifetechnologies.com) or from **Technical Support** (see page 15).



Comments for pSecTag2/PSA: 5822 nucleotides

- CMV promoter: bases 209-863
- T7 promoter/priming site: bases 863-882
- Murine Ig κ-chain V-J2-C signal peptide: bases 905-967
- PSA gene: bases 1042-1732
- c-myc* epitope: bases 1745-1777
- Polyhistidine tag: bases 1790-1807
- BGH reverse priming site: bases 1830-1847
- BGH polyadenylation sequence: bases 1829-2043
- f1 origin: bases 1954-2367
- SV40 promoter and origin: bases 2587-2908
- EM-7 promoter: bases 2924-2990
- ZeocinTM resistance gene: bases 2991-3365
- SV40 polyadenylation sequence: bases 3495-3624
- pUC origin: bases 4008-4681
- Ampicillin resistance gene: bases 4826-5686 (C)

* The *Sfi* I site is located before the variable region.

Zeocin™

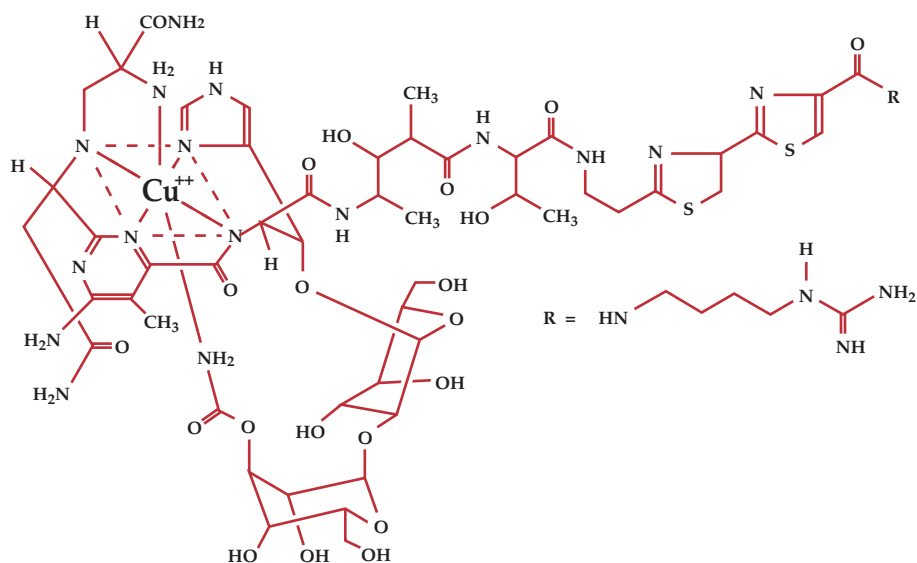
Zeocin™

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong anti-bacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells.

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ in a stoichiometric manner to inhibit its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

Molecular Weight, Formula and Structure

The formula for Zeocin™ is $C_{55}H_{86}O_{21}N_{20}S_2Cu-HCl$ and the molecular weight is 1,527.5 daltons. Zeocin™ is an HCl salt. The diagram below shows the structure of Zeocin™.



Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ for selection in mammalian cell lines and *E. coli* are listed below:

Organism	Zeocin™ Concentration and Selective Medium
<i>E. coli</i>	25–50 µg/mL in Low Salt LB medium* (see page 13 for recipe)
Mammalian Cells	50–1,000 µg/mL (varies with cell line)

*Efficient selection requires that the concentration of NaCl be no more than 5 g/liter (< 90 mM).

Recipe

Low Salt LB Medium with Zeocin™

For Zeocin™ to be active, the salt concentration of the medium must remain low (< 90 mM) and the pH must be 7.5. Prepare LB broth and plates using the following recipe. Note the lower salt content of this medium.

Failure to lower the salt content of your LB medium will result in non-selection because of inactivation of the drug.

Low Salt LB Medium:

10 g Tryptone

5 g NaCl

5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 mL. Adjust pH to 7.5 with 1 N NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
 2. Autoclave on liquid cycle at 15 lbs./sq. in. and 121°C for 20 minutes.
 3. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/mL final concentration.
 4. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1–2 weeks.
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Accessory Products

Introduction

The following products may be used with the pSecTag2 vectors. For details, visit www.lifetechnologies.com or contact **Technical Support** (page 15).

Item	Amount	Catalog no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15
Electrocomp™ TOP10F'	5 × 80 µL	C665-55
Max Efficiency® DH10B™ (chemically competent cells)	5 × 0.2 mL	18297-010
PureLink® HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink® HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Lipofectamine® 2000 Reagent	0.75 mL	11668-027
Zeocin™	1 gram	R250-01
	5 grams	R250-05
imMedia™ Zeo Liquid	200 mL	Q620-20
imMedia™ Zeo Agar	20 each	Q621-20

Antibodies

If you do not have an antibody specific to your protein, Life Technologies offers the Anti-*myc*, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-conjugated antibodies are available for convenient one-step detection.

Antibody	Epitope	Catalog no.
Anti- <i>myc</i>	Detects a 10 amino acid epitope derived from <i>c-myc</i> (Evan <i>et al.</i> , 1985): EQKLISEEDL	R950-25
Anti- <i>myc</i> -HRP		R951-25
Anti- <i>myc</i> -AP		R952-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner <i>et al.</i> , 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

Primers

For your convenience, Life Technologies offers a custom primer synthesis service. Visit www.lifetechnologies.com for more details.

Technical Support

Obtaining support For the latest services and support information for all locations, go to www.lifetechnologies.com.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Sata Sheets (SDS)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited warranty

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References

- Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989). Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. *J. Biol. Chem.* *264*, 8222-8229.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). *Current Protocols in Molecular Biology* (New York: Greene Publishing Associates and Wiley-Interscience).
- Baron, M., Reynes, J. P., Stassi, D., and Tiraby, G. (1992). A Selectable Bifunctional β -Galactosidase: Phleomycin-resistance Fusion Protein as a Potential Marker for Eukaryotic Cells. *Gene* *114*, 239-243.
- Berdy, J. (1980) Bleomycin-Type Antibiotics. In *Amino Acid and Peptide Antibiotics*, J. Berdy, ed. (Boca Raton, FL: CRC Press), pp. 459-497.
- Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. *Cell* *41*, 521-530.
- Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. *Mol. Cell. Biol.* *7*, 2745-2752.
- Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. *Nuc. Acids Res.* *15*, 1311-1326.
- Coloma, M. J., Hastings, A., Wims, L. A., and Morrison, S. L. (1992). Novel Vectors for the Expression of Antibody Molecules Using Variable Regions Generated by Polymerase Chain Reaction. *J. Imm. Methods* *152*, 89-104.
- Drocourt, D., Calmels, T. P. G., Reynes, J. P., Baron, M., and Tiraby, G. (1990). Cassettes of the *Streptoalloteichus hindustanus ble* Gene for Transformation of Lower and Higher Eukaryotes to Phleomycin Resistance. *Nuc. Acids Res.* *18*, 4009.
- Evan, G. I., Lewis, G. K., Ramsay, G., and Bishop, V. M. (1985). Isolation of Monoclonal Antibodies Specific for *c-myc* Proto-oncogene Product. *Mol. Cell. Biol.* *5*, 3610-3616.
- Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. *Proc. West. Pharmacol. Soc.* *32*, 115-121.
- Felgner, P. L., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. *Nature* *337*, 387-388.
- Goodwin, E. C., and Rottman, F. M. (1992). The 3'-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. *J. Biol. Chem.* *267*, 16330-16334.
- Lindner, P., Bauer, K., Krebber, A., Nieba, L., Kremmer, E., Krebber, C., Honegger, A., Klinger, B., Mocikat, R., and Pluckthun, A. (1997). Specific Detection of His-tagged Proteins With Recombinant Anti-His Tag scFv-Phosphatase or scFv-Phage Fusions. *BioTechniques* *22*, 140-149.
- Mulsant, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1988). Phleomycin Resistance as a Dominant Selectable Marker in CHO Cells. *Somat. Cell Mol. Genet.* *14*, 243-252.
- Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987). Negative and Positive Regulation by a Short Segment in the 5'-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. *Mol. Cell. Biol.* *7*, 4125-4129.
- Perez, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1989). Phleomycin Resistance as a Dominant Selectable Marker for Plant Cell Transformation. *Plant Mol. Biol.* *13*, 365-373.

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References, continued

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. *BioTechniques* 6, 742-751.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. *Cell* 11, 223-232.

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Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

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