

pAc5.1/V5-His A, B, & C Vectors

USER GUIDE

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Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008

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Contents

■	CHAPTER 1	Product information	5
		Product description	5
		Description of Ac5 promoter	5
		Contents and storage	6
		Experimental outline	6
■	CHAPTER 2	Methods	7
		Cloning into pAc5.1/V5-His A, B, and C	7
		General molecular biology techniques	7
		<i>E. coli</i> strain	7
		Transformation method	7
		Maintaining plasmids	7
		Cloning considerations	8
		Multiple cloning site of pAc5.1/V5-His A	9
		Multiple cloning site of pAc5.1/V5-His B	10
		Multiple cloning site of pAc5.1/V5-His C	11
		<i>E. coli</i> transformation	12
		Preparing a glycerol stock	12
		Transfection and analysis	12
		Plasmid preparation	13
		Positive control	13
		Assay for β -galactosidase activity	13
		Detection and purification of recombinant Fusion [™] proteins	13
■	APPENDIX A	Vector information	14
		About the pAc5.1/V5-His A, B, & C vectors	14
		About the pAc5.1/V5-His/lacZ vector	15
■	APPENDIX B	Accessory products	16
		Additional products	16
		Detection of recombinant proteins	17
		Purification of recombinant protein	17

- **APPENDIX C Safety 18**
 - Chemical safety 19
 - Biological hazard safety 20
- Documentation and support 21**
 - Customer and technical support 21
 - Limited product warranty 21



Product information

Product description

pAc5.1/V5-His is a 5.4 kb expression vector designed for use in *Drosophila* cells to achieve high-level transient expression of recombinant proteins. The vector can be used with the *Drosophila* Inducible Expression System (DES™). Upon transfection, the vector allows transient expression of your protein of interest in *Drosophila* cells. When cotransfected with the selection vector, pCoHygro or pCoBlast, pAc5.1/V5-His allows selection of stable cell lines exhibiting constitutive expression of the protein of interest. The pAc5.1/V5-His vector contains the following elements:

- The *Drosophila* actin 5C (Ac5) promoter for high-level, constitutive expression of the gene of interest in S2 cells (Chung and Keller, 1990)
- Multiple cloning site to facilitate cloning the gene of interest
- C-terminal peptide containing the V5 epitope and polyhistidine (6xHis) tag for detection and purification of your protein of interest (if desired)
- Three reading frames to facilitate in-frame cloning with the C-terminal peptide
- Ampicillin resistance gene for selection of transformants in *E. coli*

The control plasmid, pAc5.1/V5-His/*lacZ*, is included for use as a positive control for transfection and expression.

For more information about the DES™ Inducible Kits, pCoHygro, and pCoBlast, refer to the *Drosophila* Expression System manual. The *Drosophila* Expression System manual is available for downloading from www.lifetechnologies.com/support or by contacting Technical Support ().

Description of Ac5 promoter

The *Drosophila* Ac5 promoter allows high-level, constitutive expression of the gene of interest in *Drosophila* S2 cells. Expression levels are comparable to the induced levels obtained from the metallothionein promoter. The Ac5 promoter is well characterized (Chung and Keller, 1990) and the start of transcription is shown in the diagrams in “Multiple cloning site of pAc5.1/V5-His A” on page 9–“Multiple cloning site of pAc5.1/V5-His C” on page 11.

Note: pAc5.1/V5-His is an improved version of pAc5/V5-His. During construction of the original vector, an ATG was inadvertently created upstream of the multiple cloning site. Since it may interfere with correct translation of the cloned gene, this ATG was removed to create pAc5.1/V5-His.

Contents and storage

Vector	Buffer composition	Amount	Storage ^[1]
pAc5.1/V5-His A, B, and C (0.5 µg/µL)	10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg (40 µL)	–20°C
pAc5.1/V5-His/lacZ (0.5 µg/µL)			

^[1] Vectors are shipped at room temperature. Upon receipt, store as instructed.

Experimental outline

The table below describes the general steps needed to clone and express your gene of interest. For more details, refer to the manual and pages indicated.

Step	Action	Source
1	Develop a cloning strategy to ligate your gene of interest into pAc5.1/V5-His A, B, or C in frame with the C-terminal peptide encoding the V5 epitope and the polyhistidine tag (if desired).	“Cloning considerations” on page 8–“Multiple cloning site of pAc5.1/V5-His C” on page 11, this manual
2	Transform your ligation reactions into a <i>recA</i> , <i>endA</i> <i>E. coli</i> strain (e.g. TOP10). Select on LB agar plates containing 50–100 µg/mL ampicillin.	“E. coli transformation” on page 12, this manual
3	Analyze your transformants for the presence of insert by restriction digest.	“E. coli transformation” on page 12, this manual
4	Select a transformant with the correct restriction pattern and sequence it to confirm that your gene is cloned in frame with the C-terminal peptide.	“E. coli transformation” on page 12, this manual
5	Transfect your pAc5.1/V5-His construct into S2 cells.	“Transfection and analysis” on page 12, this manual and DES [™] manual
6	Assay for transient expression of your recombinant protein.	“Detection and purification of recombinant Fusion [™] proteins” on page 13, this manual and DES [™] manual
7	To generate stable cell lines, cotransfect your pAc5.1/V5-His construct and pCoHygro or pCoBlast into S2 cells and select for hygromycin resistant clones.	DES [™] manual
8	Scale up expression for purification.	DES [™] manual
9	Purify your recombinant protein by chromatography on metal-chelating resin (i.e. ProBond [™]).	DES [™] manual



Methods

Cloning into pAc5.1/V5-His A, B, and C

Diagrams are provided in “Multiple cloning site of pAc5.1/V5-His A” on page 9– “Multiple cloning site of pAc5.1/V5-His C” on page 11 to help you clone your gene of interest into pAc5.1/V5-His. General considerations for cloning and transformation are discussed below.

General molecular biology techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *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 propagation and maintenance of the pAc5.1/V5-His vectors including TOP10 or DH5 α [™]-T1^R. We recommend that you propagate the vectors in *E. coli* strains that are recombination deficient (*recA*) and endonuclease A deficient (*endA*).

For your convenience, TOP10 and DH5 α [™]-T1^R *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot[™] format (see Appendix B, “Accessory products” for ordering).

Transformation method

You may use any method of your choice for transformation. Chemical transformation is the most convenient method for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintaining plasmids

To propagate and maintain the pAc5.1/V5-His and pAc5.1/V5-His/*lacZ* vectors, we recommend using the supplied 0.5 μ g/ μ L stock solution in TE buffer, pH 8.0 to transform a *recA*, *endA* *E. coli* strain like TOP10, DH5 α [™]-T1^R, or equivalent. Select transformants on LB agar plates containing 50-100 μ g/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see “Preparing a glycerol stock” on page 12).

Cloning considerations

Consider the following points when designing a strategy to clone your gene of interest into pAc5.1/V5-His.

- Your insert should contain a Kozak translation initiation sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Note that other sequences are possible, but the G or A at position –3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

(G/A)NNATGG

- It is possible to clone and express a secreted protein in pAc5.1/V5-His if your protein includes a native signal sequence. If your protein does not have a secretion signal, you may wish to consider using the pMT/BiP/V5-His vector for secreted expression. For more information, refer to the DES™ manual.
- If you wish to use the V5 epitope and the polyhistidine (6xHis) tag for detection and purification of your recombinant protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. Refer to the diagrams in “Multiple cloning site of pAc5.1/V5-His A” on page 9–“Multiple cloning site of pAc5.1/V5-His C” on page 11 to develop a cloning strategy. **Be sure that your gene does not contain a stop codon upstream of the C-terminal peptide.**
- If you do not wish to include the C-terminal peptide, include the native stop codon for your gene of interest.

Multiple cloning site of pAc5.1/V5-His A

Below is the multiple cloning site for pAc5.1/V5-His A. The start of transcription can occur at either nucleotide 2,478, 2,479, or 2,480. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pAc5.1/V5-His A is available for downloading from www.thermofisher.com. For a map and a description of the features of pAc5.1/V5-His A, see “About the pAc5.1/V5-His A, B, & C vectors” on page 14.

```

2398      TGAGAGCGGA GAGCATTGCG GCTGATAAGG TTTTAGCGCT AAGCGGGCTT TATAAAACGG
                                     TATA box
2458      GCTGCGGGAC CAGTTTTCAT ATCACTACCG TTGAGTTCT TGTGCTGTGT GGATACTCCT
                                     Start of Transcription
                                     |||
                                     Ac5 Forward priming site
2518      CCCGACACAA AGCCGCTCCA TCAGCCAGCA GTCGTCTAAT CCAGAGACCC CGGATC GG

2576      Asp718 I Kpn I      BstX I EcoR I      EcoR V      BstX I Not I
      GGT ACC TAC TAG TCC AGT GTG GTG GAA TTC TGC AGA TAT CCA GCA CAG TGG CGG
      Gly Thr Tyr *** Ser Ser Val Val Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg

2630      Xho I      Xba I      Apa I BstB I      V5 epitope
      CCG CTC GAG TCT AGA GGG CCC TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC
      Pro Leu Glu Ser Arg Gly Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu

2684      Polyhistidine region      Pme I
      GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCC
      Gly Leu Asp Ser Thr Arg Thr Gly His His His His His His ***

2739      BGH Reverse priming site
      GCTGATCAGC CTCGACTGTG CTTTCTAAGG CCTGAGCTCG CTGATCAGCC TCGATCGAGG

2799      ATCCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC CACAACTAGA ATGCAGTGAA

2859      AAAAAATGCTT TATTTGTGAA ATTTGTGATG CTATTGCTTT ATTTGTAAAC ATTATAAGCT

2919      SV40 polyadenylation signal
      GCAATAAACA AGTTAACAAC AACAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG

```

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

Multiple cloning site of pAc5.1/V5-His B

Below is the multiple cloning site for pAc5.1/V5-His B. The start of transcription can occur at either nucleotide 2,478, 2,479, or 2,480. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pAc5.1/V5-His B is available for downloading from www.thermofisher.com. For a map and a description of the features of pAc5.1/V5-His B, see “About the pAc5.1/V5-His A, B, & C vectors” on page 14.

```

2399      TGAGAGCGGA GAGCATTGCG GCTGATAAGG TTTTAGCGCT AAGCGGGCTT TATAAAACGG
                                         TATA box
                                         TATAAAACGG

2459      GCTGCGGGAC CAGTTTTCAT ATCACTACCG TTTGAGTTCT TGTGCTGTGT GGATACTCCT
                               Start of Transcription
                               |||
                               |>

2519      CCCGACACAA AGCCGCTCCA TCAGCCAGCA GTCGTCTAAT CCAGAGACCC CGGATC GGG
                                         Gly

2578      Asp718 I Kpn I          BstX I EcoR I          EcoR V          BstX I Not I
          GTA CCT ACT AGT CCA GTG TGG TGG AAT TCT GCA GAT ATC CAG CAC AGT GGC GGC
          Val Pro Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His Ser Gly Gly

2632      Xho I   Xba I       Apa I Sac II BstB I       V5 epitope
          CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC
          Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu

2686      Polyhistidine region
          CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC CAT TGA
          Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His His His His ***

2734      Pme I          BGH Reverse priming site
          GTTTAAACCC GCTGATCAGC CTCGACTGTG CTTTCTAAGG CCTGAGCTCG CTGATCAGCC

2794      TCGACTGTGC CTTCTAAGGC CTGAGCTCGC TGATCAGCCT CGATCGAGGA TCCAGACATG

2854      ATAAGATACA TTGATGAGTT TGGACAAACC ACAACTAGAA TGCAGTGAAA AAAATGCTTT

2914      ATTTGTGAAA TTTGTGATGC TATTGCTTTA TTTGTAACCA TTATAAGCTG CAATAAACAA
                                         SV40 polyadenylation signal
                                         CAATAAACAA

2974      GTTAACAACA ACAATTGCAT TCATTTTATG TTTCAGGTTC AGGGGGAGGT GTGGGAGGTT

```

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

Multiple cloning site of pAc5.1/V5-His C

Below is the multiple cloning site for pAc5.1/V5-His C. The start of transcription can occur at either nucleotide 2,478, 2,479, or 2,480. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. The nucleotide sequence of pAc5.1/V5-His C is available for downloading from www.thermofisher.com. For a map and a description of the features of pAc5.1/V5-His C, “About the pAc5.1/V5-His A, B, & C vectors” on page 14.

```

2399      TGAGAGCGGA GAGCATTGCG GCTGATAAGG TTTTAGCGCT AAGCGGGCTT TATAAACGG
                                     TATA box
2459      GCTGCGGGAC CAGTTTTCAT ATCACTACCG TTTGAGTTCT TGTGCTGTGT GGATACTCCT
                                     Start of Transcription
2519      CCCGACACAA AGCCGCTCCA TCAGCCAGCA GTCGTCTAAT CCAGAGACCC CGGATCG GGG
                                     Ac5 Forward priming site
                                     Asp718 I
                                     Gly
2579      TAC CTA CTA GTC CAG TGT GGT GGA ATT CTG CAG ATA TCC AGC ACA GTG GCG GCC
          Tyr Leu Leu Val Gln Cys Gly Gly Ile Leu Gln Ile Ser Ser Thr Val Ala Ala
          Kpn I      BstX I EcoR I      EcoR V      BstX I Not I
2633      GCT CGA GGT CAC CCA TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC
          Ala Arg Gly His Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu
          Xho I BstE II BstB I      V5 epitope
2687      GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC CAT TGA GTTTAAACCCGC
          Asp Ser Thr Arg Thr Gly His His His His His His ***
          Polyhistidine region      Pme I
2738      TGATCAGCCT CGACTGTGCC TTCTAAGGCC TGAGCTCGCT GATCAGCCTC GATCGAGGAT
          BGH Reverse priming site
2798      CCAGACATGA TAAGATACAT TGATGAGTTT GGACAAACCA CAACTAGAAT GCAGTGAAAA
2858      AAATGCTTTA TTTGTGAAAT TTGTGATGCT ATTGCTTTAT TTGTAACCAT TATAAGCTGC
          SV40 polyadenylation signal
2918      AATAACAAG TTAACAACAA CAATTGCATT CATTATATGT TTCAGGTTC GGGGGAGGTG

```

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

E. coli transformation

Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10, DH5 α [™]-T1^R) and select on LB agar plates containing 50–100 μ g/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

Note: We recommend that you sequence your construct with the Ac5 Forward and BGH Reverse primers (see Appendix B, “Accessory products” for ordering) to confirm that your gene is in the correct orientation for expression and is cloned in frame with the C-terminal peptide. Refer to the diagram in “Multiple cloning site of pAc5.1/V5-His A” on page 9–“Multiple cloning site of pAc5.1/V5-His C” on page 11 for the sequences and location of the priming sites.

Preparing a glycerol stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at 20°C.

1. Streak the original colony out on an LB plate containing 50 μ g/mL ampicillin. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 μ g/mL ampicillin.
3. Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Transfection and analysis

Once you have cloned your gene of interest into pAc5.1/V5-His and have prepared purified plasmid DNA, you are ready to transfect your construct into S2 cells.

If you are assaying for transient expression of your gene of interest, you may transfect your pAc5.1/V5-His construct alone into S2 cells. If you wish to generate stable cell lines, you **must** cotransfect your pAc5.1/V5-His construct and pCoHygro or pCoBlast into S2 cells. Note that the pAc5.1/V5-His vector does not contain a resistance marker for selection in *Drosophila* cells.

We recommend that you include the pAc5.1/V5-His/*lacZ* positive control vector and a mock transfection (negative control) in your experiments to evaluate your results. Specific guidelines and protocols for transient transfection and generation of stable cell lines can be found in the DES[™] manual.

Note: Either pCoHygro or pCoBlast is supplied with the appropriate DES[™] Inducible Kits. For detailed information about each vector, refer to the DES[™] manual.

Plasmid preparation

Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure MiniPrep™ Kit (up to 30 µg DNA), the PureLink™ HiPure MidiPrep Kit (up to 150 µg DNA) (see Appendix B, “Accessory products” for ordering), or CsCl gradient centrifugation.

Positive control

pAc5.1/V5-His/lacZ is provided as a positive control vector for *Drosophila* cell transfection and expression (for a map, see “About the pAc5.1/V5-His/lacZ vector” on page 15) and may be used to optimize transfection conditions for S2 cells. Transfection of pAc5.1/V5-His/lacZ results in constitutive β-galactosidase expression. A successful transfection will result in β-galactosidase expression that can be easily assayed by staining with X-gal (see “Assay for β-galactosidase activity” on page 13).

Assay for β-galactosidase activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. The β-Gal Assay Kit and the β-Gal Staining Kit are available for fast and easy detection of β-galactosidase expression (see Appendix B, “Accessory products” for ordering).

Detection and purification of recombinant Fusion™ proteins

If you have cloned your gene of interest in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use the Anti-V5 antibodies or Anti-His(C-term) antibodies to detect expression of your recombinant fusion protein by western blot analysis (see Appendix B, “Accessory products” for ordering). The 6xHis tag also allows purification of recombinant protein using metal-chelating resins including ProBond™. Refer to the DES™ manual for more detailed guidelines and instructions to detect and purify your recombinant fusion protein.



Vector information

About the pAc5.1/V5-His A, B, & C vectors

The following table summarizes the features of pAc5.1/V5-His A (5,375 bp), pAc5.1/V5-His B (5,379 bp), and pAc5.1/V5-His C (5,371 bp). To obtain the complete nucleotide sequence for these vectors, go to www.thermofisher.com.

Bases	Description	Map
45–2,565	Drosophila actin 5C (Ac5) promoter: Permits high-level, constitutive expression of heterologous proteins	
2,448–2,453	TATA box	
2,478, 2,479, or 2,480	Start of transcription	
2,522–2,541	Ac5 Forward priming site: Allows sequencing in the sense orientation	
2,576–2,6507	Multiple cloning site: Allows insertion of your gene of interest	
2,657–2,698	V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro- Leu-Leu-Gly-Leu-Asp-Ser-Thr): Allows detection of your recombinant protein with the Anti-V5 Antibodies available.	
2,650–5,661	Polyhistidine region: Polyhistidine (6xHis) tag. Permits purification of your recombinant protein on metal-chelating resin.	
2,748–2,765	BGB Reverse priming site: Permits sequencing of the non-coding strand	
2,921–2,926	SV40 Polyadenylation signal: Allows efficient transcription termination and polyadenylation of mRNA.	
3,489–4,147 (C ^[1])	pUC origin of replication (<i>ori</i>): Permits high-copy replication and maintenance in <i>E. coli</i> .	
5,153–5,251 (C ^[1])	<i>bla</i> promoter: Allows expression of the ampicillin (<i>bla</i>) resistance gene.	<p>*Frame-dependent variations. <i>Xba I</i> and <i>Apa I</i> are found only in versions A and B. <i>Sac II</i> is found only in version B. <i>BstE II</i> is found only in version C.</p>
4,292–5,152 (C ^[1])	Ampicillin (<i>bla</i>) resistance gene: Allows selection of the plasmid in <i>E. coli</i> .	

^[1] C = Complementary strand

About the pAc5.1/V5-His/lacZ vector

pAc5.1/V5-His/lacZ is a 8,433 bp control vector expressing β -galactosidase. The plasmid was constructed by ligating a 3.2 kb *Kpn* I–*Age* I fragment containing the *lacZ* gene and the V5 epitope in frame with the polyhistidine tag into pAc5.1/V5-His B. The following table summarizes the features of the pAc5.1/V5-His/lacZ vector. To obtain the complete nucleotide sequence for the pAc5.1/V5-His/lacZ vector, go to www.thermofisher.com.

Bases	Description	Map
45–2,565	Drosophila actin 5C (Ac5) promoter: Permits high-level, constitutive expression of heterologous proteins	
2,448–2,453	TATA box	
2,478, 2,479, or 2,480	Start of transcription	
2,522–2,541	Ac5 Forward priming site: Allows sequencing in the sense orientation	
2,631–5,687	β galactosidase portion of fusion	
5,715–5,756	V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro- Leu-Leu-Gly-Leu-Asp-Ser-Thr): Allows detection of your recombinant protein with the Anti-V5 Antibodies available.	
5,766–5,783	Polyhistidine region: Polyhistidine (6xHis) tag. Permits purification of your recombinant protein on metal-chelating resin.	
5,806–5,823	BGB Reverse priming site: Permits sequencing of the non-coding strand	
5,979–5,984	SV40 Polyadenylation signal: Allows efficient transcription termination and polyadenylation of mRNA.	
6,566–7,226 (C ^[1])	pUC origin of replication (<i>ori</i>): Permits high-copy replication and maintenance in <i>E. coli</i> .	
8,232–8,330 (C ^[1])	<i>bla</i> promoter: Allows expression of the ampicillin (<i>bla</i>) resistance gene.	
7,371–8,231 (C ^[1])	Ampicillin (<i>bla</i>) resistance gene: Allows selection of the plasmid in <i>E. coli</i> .	

^[1] C = Complementary strand



Accessory products

Additional products

Reagents suitable for use with the product are available separately. Ordering information for these reagents is provided below. For more information, refer to www.lifetechnologies.com.

Item	Amount	Catalog no.
One Shot™ TOP10 Chemically Competent <i>E. coli</i>	21 × 50 µL	C4040-03
One Shot™ TOP10 Electrocomp™ <i>E. coli</i>	21 × 50 µL	C4040-52
One Shot™ DH5α™ -T1 ^R Max™ Efficiency Chemically Competent <i>E. coli</i>	21 × 50 µL	12297-016
PureLink™ HiPure MiniPrep™ Kit	25 preps	K2100-02
PureLink™ HiPure MidiPrep Kit	25 preps	K2100-04
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
BGH Reverse Primer	2 µg, lyophilized in TE	N575-02
Blasticidin S HCl	50 mg	R210-01
Schneider (S2) Cells	1 mL vial, 1 × 10 ⁷ cells/mL	R690-07
Schneider's Drosophila Medium	500 mL	11720-034
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
DES™ Inducible Kit	1 Kit	K4120-01
with <i>pCoHygro</i>	1 Kit	K5120-01
with <i>pCoBlast</i>		
DES™ -Inducible/Secreted Kit	1 Kit	K4130-01
with <i>pCoHygro</i>	1 Kit	K5130-01
with <i>pCoBlast</i>		



Detection of recombinant proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for detection of C-terminal fusion proteins expressed using pAc5.1/V5-His. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

The amount of antibody supplied is sufficient for 25 westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus SV5 GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

Purification of recombinant protein

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using the ProBond™ Resin (see below). To purify proteins expressed from pAc5.1/V5-His, the ProBond™ Purification System or the ProBond™ resin in bulk are available separately. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 mL	R801-01
	150 mL	R801-15
ProBond™ Purification System (includes six 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification)	6 purifications	K850-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
Purification Columns (10 mL polypropylene columns)	50	R640-50



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
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Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
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Documentation and support

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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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