IBA Headquarters
IBA GmbH
Rudolf-Wissell-Str. 28
37079 Goettingen
Germany
Tel. +49 (0) 551-5 06 72-0
Fax +49 (0) 551-5 06 72-181

IBA US Distribution Center
1328 Ashby Road
Olivette, MO 63132
USA
Tel. 1-877-IBA-GmbH
(1-877-422-4624)
Fax 1-888-531-6813

E-mail: info@iba-lifesciences.com
http://www.iba-lifesciences.com

Data Sheet

pASK-IBA16

Cat. No.: 2-1315-000
Lot No.: 1315 -

Last date of revision
March 2012
Version 1315-4

Description	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be secreted into the periplasm.
Affinity tag	*Strep*-Tactin® affinity tag (*Strep*-tag II®) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein and can be removed by cleavage with TEV protease (tabacco etch virus). TEV protease is a site-specific protease with a seven amino acid recognition site (in pASK-IBA16: ENLYFQG) and cleavage occurs between glutamine (Q) and glycine (G).
Bacterial Expression	Expression is induced upon addition of 200 µg anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter *E. coli* shaking culture (A_{550} = 0.5).
Expression strain	Any *E. coli* strain. The *tet*-promoter works independently from the genetic background of *E. coli*.
Resistance	Ampicillin
Form	5 µg, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 µl
Concentration	250 ng/µl
Storage	4 °C for frequent usage, -20 °C for long-term storage

For research use only

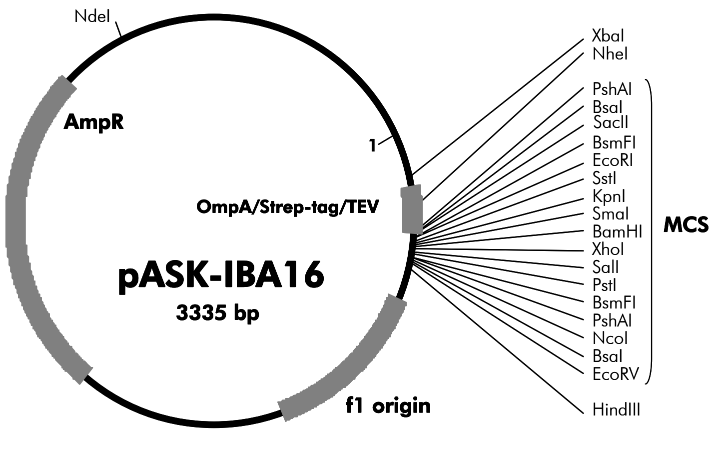
Multiple Cloning Site of pASK-IBA16

```
1     CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                 M  K  K  T  A  I  A
81    GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA   160
                                           XbaI

                   OmpA                      link       Strep-tag          linker
      I  A  V  A  L  A  G  F  A  T  V  A  Q  A  A  S  W  S  H  P  Q  F  E  K  S  G  G
161   TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCTGGTGGT   240
                                              NheI

                                           D  R  G  P  E  F  E  L  G  T  R  G  S  L  E
                 TEV protease            R  P  R  S  R  I  R  A  R  Y  P  G  I  P  R
       G  G  G  E  N  L  Y  F  Q  G  A  E  T  A  V  P  N  S  S  S  V  P  G  D  P  S  R
241   GGTGGTGGTGAGAATCTTTATTTTCAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAG   320
                                   BbeI  BsaI     BsmFI    SstI  KpnI    BamHI
                                   EheI   PshAI        EcoRI          SmaI      XhoI
                                   KasI     SacII
                                   NarI

       V  D  L  Q  G  D  H  G  L  *
      G  R  P  A  G  G  P  W  S  L  I  S  N  *
        S  T  C  R  G  T  M  V  S  D  I  *
321   GTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGA   400
      SalI  PstI  BsmFI  BsaI  EcoRV     HindIII
                   PshAI
                     NcoI

401   CATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGT   480
                        reverse primer
```

Please note: Restriction enzymes in bold cut twice. The *Bsa*I sites (isoschizomer of *Eco31*I) at each end of the multiple cloning site are useful for precise and oriented insertion of the recombinant gene by one cleavage reaction only. The "link" contains a restriction site which can be used e.g. for subcloning the recombinant gene into pEXPR-IBA vectors for mammalian expression. During secretion of the recombinant protein into the periplasmic space, the OmpA signal sequence will be cleaved off. The processed protein will start with the Ala-Ser-linker.

Features of pASK-IBA16

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
TEV cleavage site	232	272
multiple cloning site	273	349
reverse primer binding site	417	433
f1 origin	446	884
AmpR resistance gene	1033	1893
tet-repressor	1903	2526
Col E1origin	2679	3267



Cloning primers for the precise cloning using *Bsa*I or *Eco31*I	**Sequencing primers:**
Forward: 5'- NNNNNNGGTCTCNGC GCC NNN NNN... (N_{20})	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA NNN NNN... (N_{20})	Reverse: 5'- CGCAGTAGCGGTAAACG -3'