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-IBA6

Cat. No.: 2-1305-000
Lot No.: 1305-

Last date of revision
March 2012
Version 1305-8

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 factor Xa.
Secretion	The ompA signal sequence directs the expressed protein into the periplasmic space and will be cleaved off during the translocation process
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

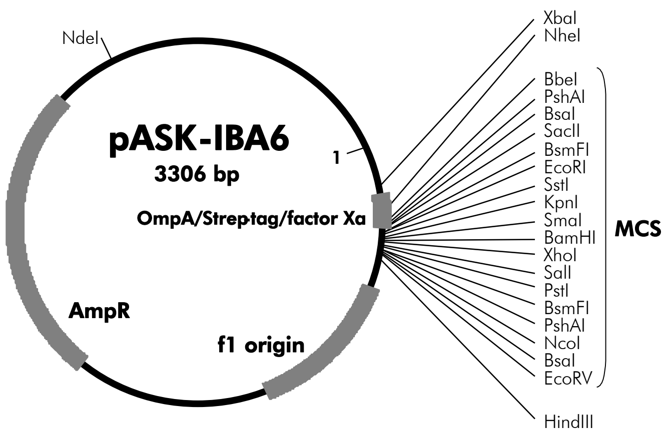
Multiple Cloning Site of pASK-IBA6

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                           forward_primer

                                                                                   OmpA
                                                                          M  K  K  T  A  I  A
81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA   160
                                                 XbaI
                   OmpA                              link       Strep-tag          factor Xa
        I  A  V  A  L  A  G  F  A  T  V  A  Q  A  A  S  W  S  H  P  Q  F  E  K  I  E  G h
161     TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAAATCGAAGGg   240
                                                    NheI                               BbeI
                                                                                       EheI
                                                                                       KasI
                                                                                       NarI

         R  R  D  R  G  P  E  F  E  L  G  T  R  G  S  L  E  V  D  L  Q  G  D  H  G  L  *
241     cgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTg   320
            BsaI      BsmFI     SstI  KpnI     BamHI      SalI  PstI  BsmFI   BsaI    EcoRV
              PshAI       EcoRI           SmaI       XhoI             PshAI
                SacII                                                   NcoI

321     ataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTAC   400
                 HindIII                                                  reverse primer

401     TGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC   480
```

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-IBA6

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
factor Xa cleavage site	232	243
multiple cloning site	244	320
reverse primer binding site	388	404
f1 origin	417	855
AmpR resistance gene	1004	1864
tet-repressor	1874	2497
Col E1origin	2650	3238



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