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-IBA3C

Cat. No.: 2-1322-000
Lot No.: 1322-

Last date of revision
March 2012
Version 1322-9

Description	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm.
Affinity tag	*Strep*-Tactin® affinity tag (*Strep*-tag II®) for the purification of recombinant protein. The affinity tag is fused to the C-terminus of the recombinant protein.
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	Chloramphenicol **Note:** The CamR resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of E.coli transformed with this plasmid
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

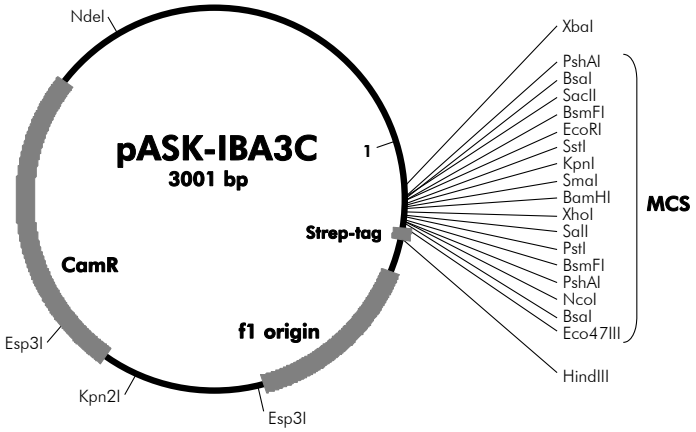
Multiple Cloning Site of pASK-IBA3C

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                 M  G  D  R  G  P  E
81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAatgGGAGACCGCGGTCCCGAAT   160
                                        XbaI                       BsaI      BsmFI
                                                                     PshAI       EcoRI
                                                                       SacII

                                                              link      Strep-tag
        F  E  L  G  T  R  G  S  L  E  V  D  L  Q  G  D  H  G  L  S  A  W  S  H  P  Q  F
161     TCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCCGCAGTTC   240
          SstI  KpnI     BamHI       SalI  PstI   BsmFI   BsaI  Eco47III
                    SmaI       XhoI                PshAI
                                                     NcoI
        E  K  *
241     GAAAAATAATAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTAC   320
                 HindIII                                                  reverse primer

321     TGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC   400
```

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.

Features of pASK-IBA3C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
multiple cloning site	139	222
Strep-tag	223	252
reverse primer binding site	308	324
f1 origin	337	775
CamR resistance gene	897	1556
Tet-repressor	1569	2192
Col E1origin	2345	2933



Cloning primers for the precise cloning using *Bsa*I or *Eco31*I	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNA ATG (N_{17})* NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT (N_{20}) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'

* The ATG start codon is already included