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## Data Sheet

### pASK-IBA4C

Cat. No.: 2-1323-000

Version: 11.0  
Revision Date: 11.06.2021

<b>Description</b>	<b>Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be secreted into the periplasm.</b>
<b>Affinity tag</b>	Strep-tag®II fused to the N-terminus of the recombinant protein.
<b>Secretion</b>	The <i>ompA</i> signal sequence directs the expressed protein into the periplasmic space and will be cleaved off during the translocation process
<b>Bacterial Expression</b>	Expression is induced upon addition of 200 µg anhydrotetracycline per 1 liter <i>E. coli</i> shaking culture ( $A_{550} = 0.5$ ).
<b>Expression strain</b>	Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> .
<b>Resistance</b>	Chloramphenicol <b>Note:</b> The Cam <sup>R</sup> resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of <i>E. coli</i> transformed with this plasmid.
<b>Form</b>	5 µg, dissolved in 20 µl TE buffer, pH 8.0: 10 mM Tris/HCl, 1 mM EDTA
<b>Concentration</b>	250 ng/µl
<b>Stability</b>	12 months after shipping
<b>Storage</b>	recommended: 2-8 °C for frequent usage, -20 °C for long-term storage
<b>Shipping</b>	room temperature
<b>Hazards</b>	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

#### For research use only

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## Multiple Cloning Site of pASK-IBA4C

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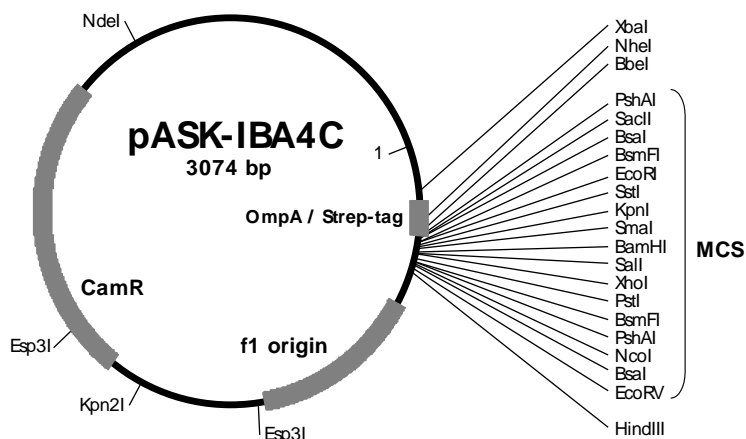
1      CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA  80
                                     forward primer
                                     M K K T A I A
81     GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA  160
                                     XbaI
                                     OmpA          link          Strep-tag®II          link R
I   A V A L A G F A T V A Q A A S W S H P Q F E K G A E
161    TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgggcCGAG  240
                                     NheI                                     BbeI  BsaI
                                     EbeI  PshAI
                                     KasI
                                     NarI
D R G P E F E L G T R G S L E V D L Q G D H G L *
P R S R I R A R Y P G I P R G R P A G G P W S L I S
T A V P N S S S V P G D P S R S T C R G T M V S D I *
241    ACCGGGTCCCGAATTCGAGCTCGGTACCCGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTA  320
      SacII  EcoRI  KpnI  BamHI  SalI  PstI  BsmFI  BsaI  EcoRV
      BsmFI  SstI  SmaI  XhoI  PshAI
                                     NcoI
N *
321    ACTAAGCTTGACCTGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTGTCTGCCGTTTACCGCTACTGCGTCA  400
      HindIII                                     reverse primer

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**Please note:** Restriction enzymes in bold cut twice. The *BsaI* sites (isoschizomer of *Eco31I*) 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 for subcloning. 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-IBA4C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag®II	202	231
multiple cloning site	232	313
reverse primer binding site	381	397
f1 origin	410	848
CamR resistance gene	970	1629
Tet-repressor	1642	2265
ColE1 origin	2418	3006



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC <sup>(N<sub>20</sub>)</sup> NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA <sup>(N<sub>20</sub>)</sup> NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'