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Data Sheet

pASK-IBA4C

Cat. No.: 2-1323-000
Lot No.: 1323-

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Version 1323-9

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.
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	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

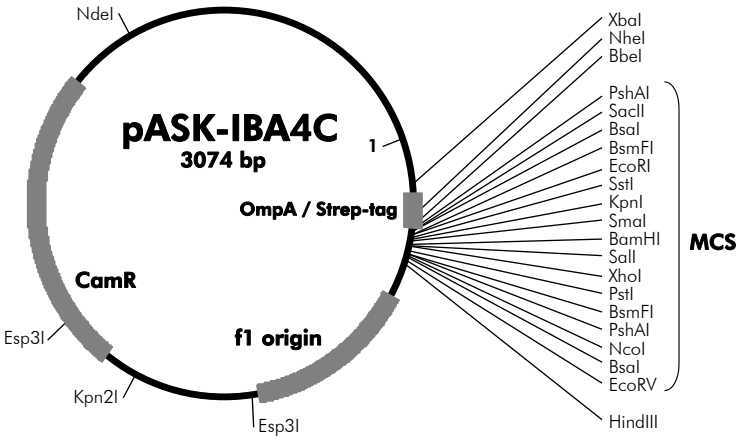
Multiple Cloning Site of pASK-IBA4C

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                 M  K  K  T  A  I  A

81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA  160
                                      XbaI

                 OmpA                                link        Strep-tag         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     TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgcCGAG  240
                                          NheI                                  BbeI  BsaI
                                                                                EheI    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     ACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTA  320
         SacII     EcoRI       KpnI     BamHI       SalI  PstI   BsmFI   BsaI   EcoRV
               BsmFI     SstI      SmaI       XhoI                 PshAI
                                                                     NcoI
        N  *

321     ACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCA  400
           HindIII                                                   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-IBA4C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	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
Col E1origin	2418	3006



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