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Data Sheet

pASK-IBA4C

Cat. No.: 2-1323-000

Version: 11.0
Revision Date: 11.06.2021

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-tag®II 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 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 Cam^R 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 20 µl TE buffer, pH 8.0: 10 mM Tris/HCl, 1 mM EDTA
Concentration	250 ng/µl
Stability	12 months after shipping
Storage	recommended: 2-8 °C for frequent usage, -20 °C for long-term storage
Shipping	room temperature
Hazards	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

For research use only

Multiple Cloning Site of pASK-IBA4C

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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                   forward primer

                                                                   M  K  K  T  A  I  A
81     GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA   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    TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgcCGAG   240
                                             NheI                             BbeI  BsaI
                                                                              EheI    PshAI
                                                                              KasI
                                                                              NarI

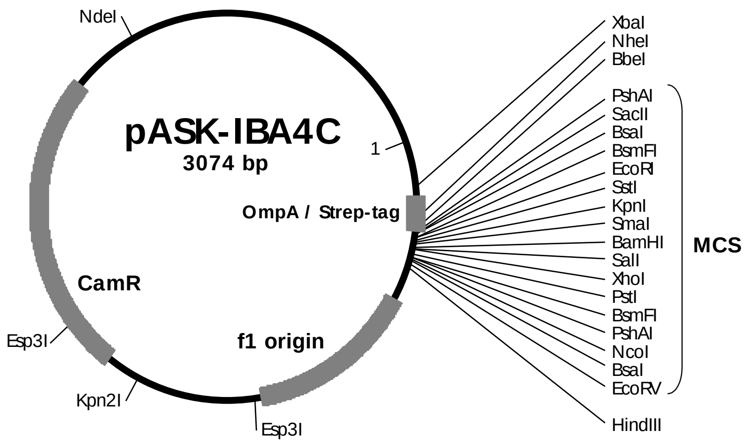
       D  R  G  P  E  F  E  L  G  T  R  G  S  L  E  V  D  L  Q  G  D  H  G  L  *
         P  R  S  R  I  R  A  R  Y  P  G  I  P  R  G  R  P  A  G  G  P  W  S  L  I  S
        T  A  V  P  N  S  S  S  V  P  G  D  P  S  R  S  T  C  R  G  T  M  V  S  D  I  *
241    ACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTA   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 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 *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'