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Data Sheet

pASK-IBA2C

Cat. No.: 2-1321-000

Version: 11.0
Revision Date: 07.01.2021

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-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.
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

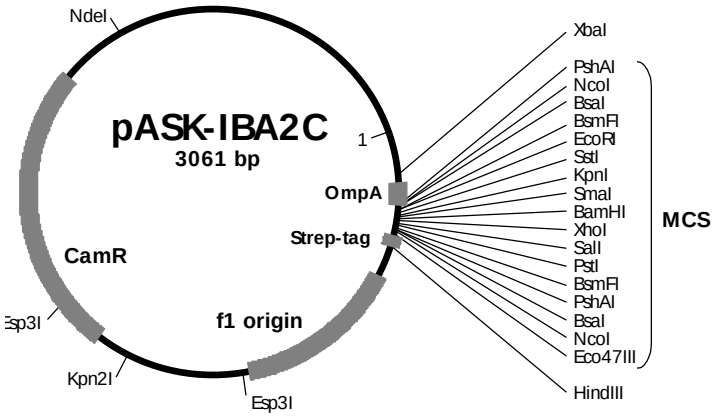
Multiple Cloning Site of pASK-IBA2C

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                        OmpA
                                                                 M  K  K  T  A  I  A

81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA  160
                                          XbaI
                    OmpA
        I  A  V  A  L  A  G  F  A  T  V  A  Q  A  G  D  H  G  P  E  F  E  L  G  T  R  G

161     TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAggccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA  240
                                          BsaI      BsmFI     SstI  KpnI     BamHI
                                           PshAI       EcoRI          SmaI
                                            NcoI

                                          link        Strep-tag®II
         S  L  E  V  D  L  Q  G  D  H  G  L  S  A  W  S  H  P  Q  F  E  K  *

241     TCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCCGCAGTTCGAAAAATAATAAGCTTGACC  320
           XhoI  SalI  PstI   BsmFI   BsaI  Eco47III                        HindIII
                             PshAI
                              NcoI

321     TGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGC  400
                                                    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. 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 first amino acid after the last Alanine of the signal sequence.

Features of pASK-IBA2C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
multiple cloning site	202	282
Strep-tag®II	283	312
reverse primer binding site	368	384
f1 origin	397	835
CamR resistance gene	957	1616
Tet-repressor	1629	2252
Col E1origin	2405	2993



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