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Data Sheet

pASK-IBA4

Cat. No.: 2-1303-000
Lot No.: 1303-

Last date of revision
March 2012
Version 1303-8

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	Ampicillin
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

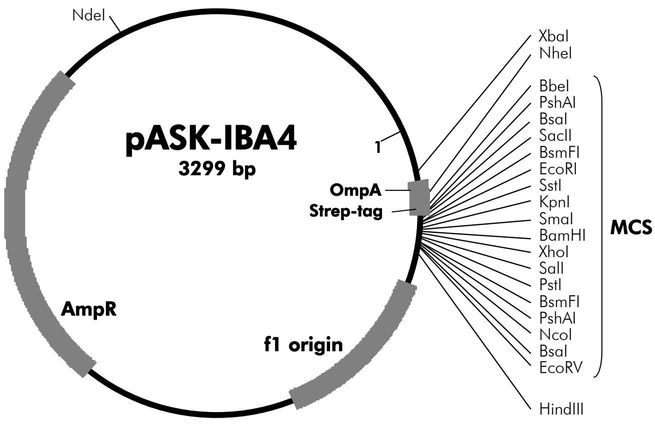
Multiple Cloning Site of pASK-IBA4

```
1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                 M  K  K  T  A  I  A
81     GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA   160
                                               XbaI

                   OmpA                         link          Strep-tag
       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

        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

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-IBA4

	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	231	313
reverse primer binding site	381	397
f1 origin	410	848
AmpR resistance gene	997	1857
tet-repressor	1867	2490
Col E1origin	2643	3231



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'