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Data Sheet

pASK-IBA44

Cat. No.: 2-1344-000
Lot No.: 1344 -

Last date of revision
March 2012
Version 1344-9

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	The recombinant protein will contain two affinity tags: **1)** *Strep*-Tactin® affinity tag (*Strep*-tag II®) for the purification of recombinant protein via *Strep*-Tactin resins. The *Strep*-tag is fused to the N-terminus of the recombinant protein. **2)** 6xHistidine-tag for the purification of recombinant protein via Ni-NTA resins. The 6xHistidine-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 (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

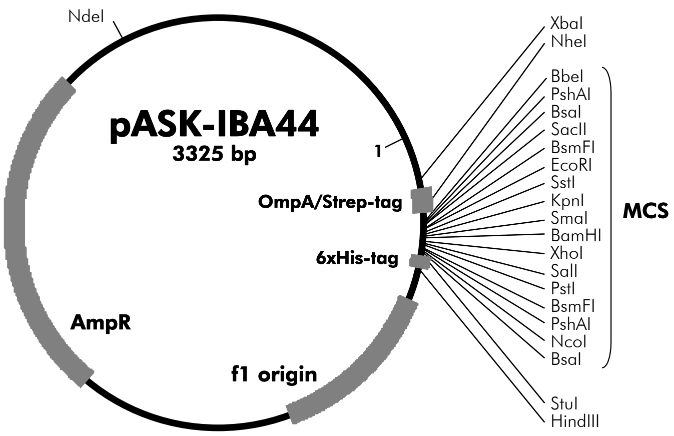
Multiple Cloning Site of pASK-IBA44

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                    forward primer

                                                                          M  K  K  T  A  I  A
81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA   160
                                                  XbaI

                   OmpA                             link        Strep-tag        link
        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  G  L  R
241     ACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAggccTGAG   320
         SacII    EcoRI      KpnI     BamHI      SalI  PstI  BsmFI   BsaI  StuI
              BsmFI     SstI      SmaI      XhoI               PshAI
                                                                  NcoI

             6xHistidine-tag
          G  S  H  H  H  H  H  H  *
321     AGGATCGCATCACCATCACCATCACTAATAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTT   400
                                          HindIII

401     GTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTA   480
              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-IBA44

	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	318
6xHistidine-tag	319	348
reverse primer binding site	407	423
f1 origin	436	874
AmpR resistance gene	1023	1883
tet-repressor	1893	2516
Col E1origin	2669	3257



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'- NNNNNNGGTCTCNG GCC NNN NNN... (N_{20})	Reverse: 5'- CGCAGTAGCGGTAAACG -3'