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Data Sheet

pASK-IBA32

Cat. No.: 2-1332-000
Lot No.: 1332 -

Last date of revision
March 2012
Version 1332-11

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	6xHistidine-tag 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 (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

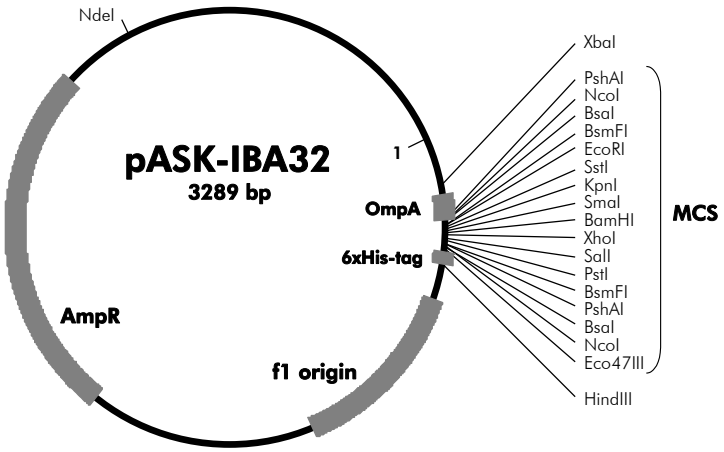
Multiple Cloning Site of pASK-IBA32

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                 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          6xHistidine-tag
         S  L  E  V  D  L  Q  G  D  H  G  L  S  A  R  G  S  H  H  H  H  H  H  *

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

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

Features of pASK-IBA32

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
multiple cloning site	202	282
6xHistidine-tag	283	312
reverse primer binding site	371	387
f1 origin	400	838
AmpR resistance gene	987	1847
tet-repressor	1857	2480
Col E1origin	2633	3221



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′