

Data Sheet

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pASK-IBA16

Cat. No. : 2-1315-000

Lot No.: 1315 -

Last date of revision
May 10

Version 1315-4

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	<i>Strep-Tactin</i> [®] affinity tag (<i>Strep-tag II</i> [®]) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein and can be removed by cleavage with TEV protease (tobacco etch virus). TEV protease is a site-specific protease with a seven amino acid recognition site (in pASK-IBA16: ENLYFQG) and cleavage occurs between glutamine (Q) and glycine (G).
Bacterial Expression	Expression is induced upon addition of 200 µg anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter <i>E. coli</i> shaking culture ($A_{550} = 0.5$).
Expression strain	Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> .
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

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Multiple Cloning Site of pASK-IBA16

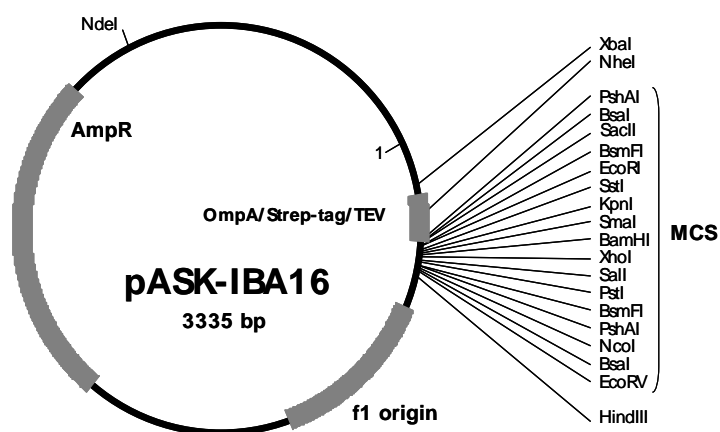
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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCCTCCCTATCA 80
      forward primer
      M K K T A I A
81     GTGATAGAGAAAAGTGAAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGCGA 160
      XbaI
      OmpA      link      Strep-tag      linker
      I A V A L A G F A T V A Q A A S W S H P Q F E K S G G
161    TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGAGTTCGAAAAATCTGGTGGT 240
      NheI
      D R G P E F E L G T R G S L E
      R P R S R I R A R Y P G I P R
      TEV protease
      G G G E N L Y F Q G A E T A V P N S S S V P G D P S R
241    GGTGGTGGTGAGAATCTTTATTTTCAGGggcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAG 320
      BbeI  BsaI  BsmFI  SstI  KpnI  BamHI
      EheI  PshAI  EcoRI  SmaI  XhoI
      KasI  SacII
      NarI
      V D L Q G D H G L *
      G R P A G G P W S L I S N *
      S T C R G T M V S D I *
321    GTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGA 400
      SalI  PstI  BsmFI  BsaI  EcoRV  HindIII
      PshAI
      NcoI
401    CATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGGGCGGGT 480
      reverse primer
  
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Please note: Restriction enzymes in bold cut twice. The *BsaI* sites (isoschizomer of *Eco31I*) 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-IBA16

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
TEV cleavage site	232	272
multiple cloning site	273	349
reverse primer binding site	417	433
f1 origin	446	884
AmpR resistance gene	1033	1893
tet-repressor	1903	2526
Col E1 origin	2679	3267



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN...	Forward: 5'- GAGTTATTTTACCCTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'