

Data Sheet

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pASK-IBA12

Cat. No. : 2-1311-000

Lot No.: 1311 -

Last date of revision
May 10

Version 1311-8

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	<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 Thrombin. The cleavage is enhanced due to a "kinker" site ¹⁾ .
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

1) Hakes, J.D. & Dixon, J.E. (1992): *Anal. Biochem.* 202, 293-298.

For research use only

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Multiple Cloning Site of pASK-IBA12

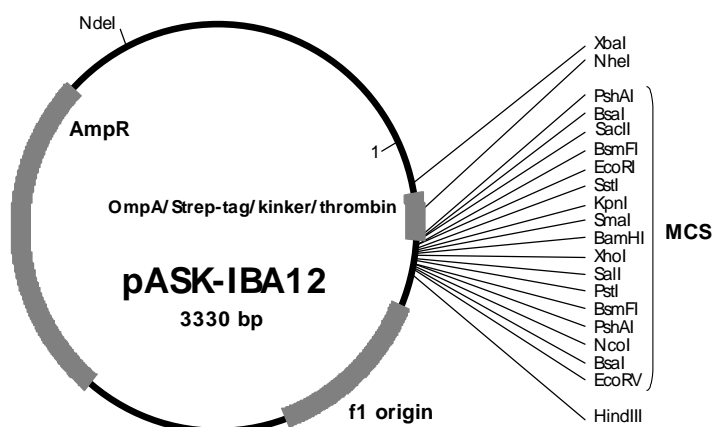
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1      CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA  80
      forward primer
      M K K T A I A
81     GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAAGACAGCTATCGCGA  160
      XbaI
      OmpA          link          Strep-tag          kinker
      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
      R P R S R I R A R Y P G I P R G R
      thrombin      E T A V P N S S S V P G D P S R S
      G G G L V P R G S R D R G P E F E L G T R G S L E V D
241    GGTGGTGGTCTGGTTCGCGTGGTccCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCTGA  320
      BsaI      BsmFI      SstI KpnI      BamHI      SalI
      PshAI      EcoRI      SmaI      XhoI
      SacII
      P A G G P W S L I S N *
      T C R G T M V S D I *
      L Q G D H G L *
321    CCTGCAGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTT  400
      PstI      BsmFI      BsaI      EcoRV      HindIII
      PshAI
      NcoI
401    TTTTGTCTGCCGTTTACCGCTACTGCGTTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGGTGTGGT  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-IBA12

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
kinker	232	249
thrombin cleavage site	250	267
multiple cloning site	268	344
reverse primer binding site	412	428
f1 origin	441	879
AmpR resistance gene	1028	1888
tet-repressor	1898	2521
Col E1 origin	2674	3262



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