

Data Sheet

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pASK-IBA32

Cat. No. : 2-1332-000

Lot No.: 1332 -

Last date of revision
May 10

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 <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-IBA32

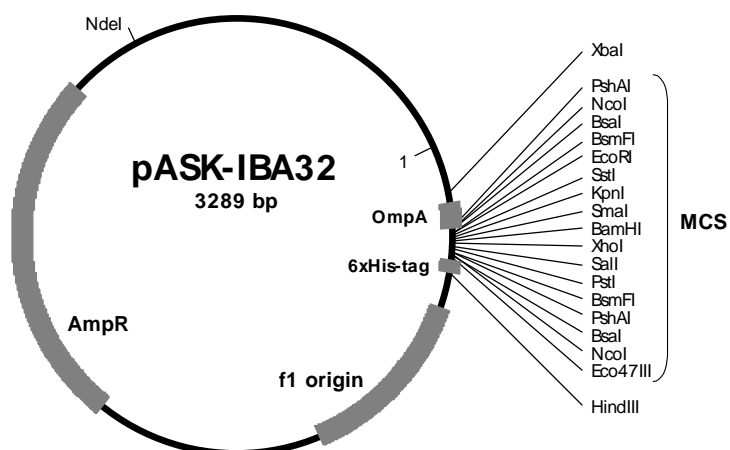
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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA 80
                                     forward primer
                                     M K K T A I A
81     GTGATAGAGAAAAGTGAAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAGACAGCTATCGCGA 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    TCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCAgcgCTAGAGGATCGCATCACCATCACCTACTAATAAGCTTG 320
       XhoI  SalI  PstI  BsmFI  BsaI  Eco47III  HindIII
       PshAI
       NcoI
321    ACCTGTGAAGTGAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCGCTTACCGCTACTGCGTACCGGATCTCCA 400
                                     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 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 E1 origin	2633	3221



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'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT ^(N₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'