

Data Sheet

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pASK-IBA35plus

Cat. No. : 2-1435-000

Lot No.: 1435-

Last date of revision
May 10

Version 1435-9

Description	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm.
Affinity tag	6xHistidine-tag for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein.
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-IBA35plus

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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTGTGTTGACACTCTATCATTGATAGAGTTAATTTTACCACTCCCTATC  79
                                     forward primer

80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACAA  159
                                     XbaI

                                     D R G P E F E L G T R G
link      6xHistidine-tag      link      R P R S R I R A R Y P G
M  A  S  R  G  S  H  H  H  H  H  H  G  A  E  T  A  V  P  N  S  S  S  V  P  G  D

160    ATGGCTAGCAGAGGATCGCATCACCATCACCATCACGgcgcCGAGACCGGGTCCCGAATTCGAGCTCGGTACCCGGGGA  239
      NheI                                     BbeI  BsaI   BsmFI   SstI  KpnI   BamHI
      EheI                                     EheI   PshAI   EcoRI                                     SmaI
      KasI                                     KasI   SacII
      NarI                                     NarI

      S L E V D L Q G D H G L *
I P R G R P A G G P W S L I S N *
P S R S T C R G T M V S D I *

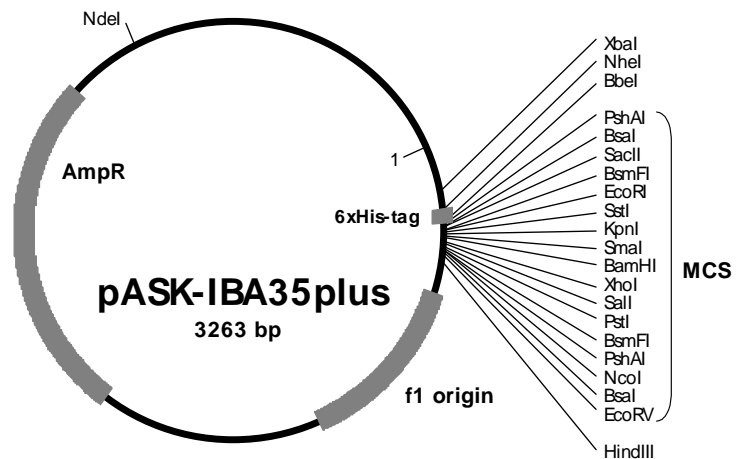
240    TCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCAC  319
      XhoI  SalI  PstI  BsmFI  BsaI  EcoRV      HindIII
      PshAI
      NcoI

320    ATTGTGCGACATTTTTTTGTCTGCCGTTTACCCTACTGCGTCACGGATCTCCACGCGCCTGTAGCGGCGCATTAAAGC  399
                                     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.

Features of pASK-IBA35plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
6xHistidine-tag	160	195
multiple cloning site	196	277
reverse primer binding site	345	361
f1 origin	374	812
AmpR resistance gene	932	1792
Tet-repressor	1802	2425
Col E1 origin	2578	3166



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'- GAGTTAATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAACG -3'