Data Sheet

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Version 1437-9

pASK-IBA37plus

Cat. No. : 2-1437-000

Lot No.: 1437-

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 and can be removed by cleavage with factor Xa.
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-IBA37plus

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1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC   79
                                                                  forward primer

80      AGTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAA  159
                                      XbaI
                                                          R  P  R  S  R  I  R  A  R  Y
           link          6xHistidine-tag      factor Xa   E  T  A  V  P  N  S  S  S  V
        M  A  S  R  G  S  H  H  H  H  H  H  I  E  G  R  R  D  R  G  P  E  F  E  L  G  T

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

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

240     CCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAAT  319
           BamHI       SalI  PstI   BsmFI   BsaI   EcoRV      HindIII
                 XhoI                 PshAI
                                       NcoI

320     GGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGC  399
                                      reverse primer
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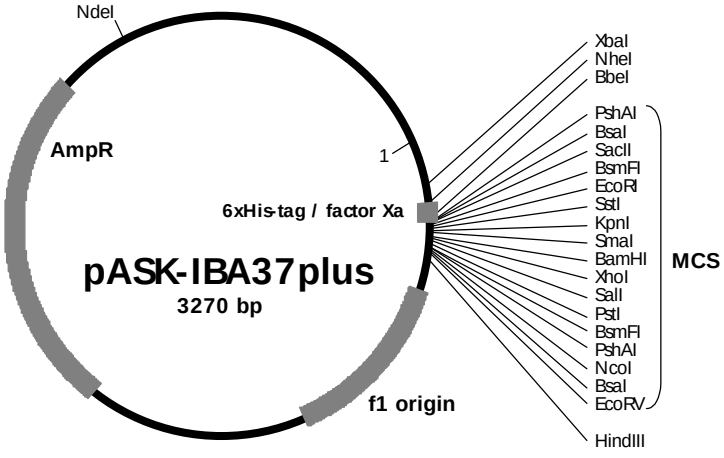
Please note: Restriction enzymes in bold cut twice. The *Bsa*I sites (isoschizomer of *Eco31*I) at each end of the multiple cloning site are useful for precise and oriented insertion of the recombinant gene by one cleavage reaction only. The "link" contains a restriction site which can be used e.g. for subcloning the recombinant gene into pEXPR-IBA vectors for mammalian expression.

Features of pASK-IBA37plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
6xHistidine-tag	160	195
factor Xa cleavage site	196	207
multiple cloning site	208	284
reverse primer binding site	352	368
f1 origin	381	819
AmpR resistance gene	968	1828
tet-repressor	1838	2461
Col E1origin	2614	3202



Cloning primers for the precise cloning using *Bsa*I or *Eco31*I	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNG CGC (N_{20}) NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA (N_{20}) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'