IBA Lifesciences GmbH
Rudolf-Wissell-Str. 28
37079 Goettingen
Germany
Tel.: +49 (0) 551-5 06 72-0
E-mail: info@iba-lifesciences.com
www.iba-lifesciences.com

Data Sheet

pASK-IBA6C

Cat. No.: 2-1325-000

Version: 11.0
Revision Date: 11.06.2021

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	Strep-tag®II fused to the N-terminus of the recombinant protein and can be removed by cleavage with Factor Xa.
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 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	Chloramphenicol **Note:** The Cam^R resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of *E. coli* transformed with this plasmid.
Form	5 µg, dissolved in 20 µl TE buffer, pH 8.0: 10 mM Tris/HCl, 1 mM EDTA
Concentration	250 ng/µl
Stability	12 months after shipping
Storage	recommended: 2-8 °C for frequent usage, -20 °C for long-term storage
Shipping	room temperature
Hazards	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

For research use only

Multiple Cloning Site of pASK-IBA6C

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1         CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTAT   78
                                                                               forward primer

                                                                               M  K  K  T  A  I  A
79        CAGTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAAGACAGCTATCGC   158
                                                    XbaI

                    OmpA                       link       Strep-tag®II      Factor Xa
           I  A  V  A  L  A  G  F  A  T  V  A  Q  A  A  S  W  S  H  P  Q  F  E  K  I  E
159       GATTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAAATCGAAG   238
                                                        NheI

                R  P  R  S  R  I  R  A  R  Y  P  G  I  P  R  G  R  P  A  G  G  P  W  S
               E  T  A  V  P  N  S  S  S  V  P  G  D  P  S  R  S  T  C  R  G  T  M  V  S
          G  R  R  D  R  G  P  E  F  E  L  G  T  R  G  S  L  E  V  D  L  Q  G  D  H  G  L
239       GgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTC   318
          BbeI  BsaI     BsmFI     SstI  KpnI    BamHI       SalI  PstI   BsmFI  BsaI
          EheI   PshAI        EcoRI         SmaI       XhoI               PshAI
          KasI     SacII                                                     NcoI
          NarI

          L  I  S  N  *
            D  I  *
           *
319       TgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCT   398
          EcoRV      HindIII                                                  reverse primer

399       ACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT   478
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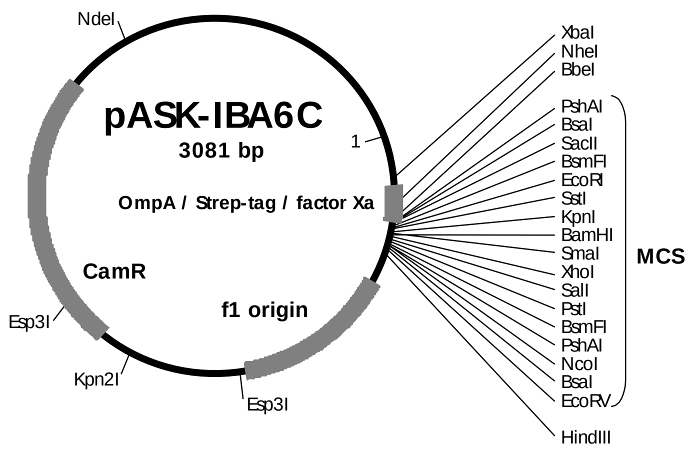
Please note: Restriction enzymes in bold cut twice. The *Bsa*I sites (isoschizomer of *Eco31*I) at each end of the multiple cloning site are useful for precise and oriented insertion of the recombinant gene by one cleavage reaction only. The "link" contains a restriction site which can be used for subcloning.

Features of pASK-IBA6C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag®II	202	231
Factor Xa cleavage site	232	243
multiple cloning site	244	320
reverse primer binding site	388	404
f1 origin	417	855
CamR resistance gene	977	1636
Tet-repressor	1649	2272
ColE1 origin	2425	3013



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