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Data Sheet

pASK-IBA7C

Cat. No.: 2-1326-000

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 localized in the cytoplasm.**
Affinity tag	Strep-tag®II 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 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

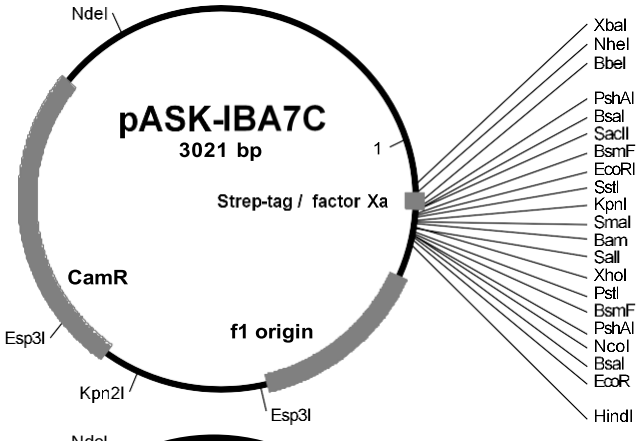
Multiple Cloning Site of pASK-IBA7C

```
1   CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA    80
                                                          forward primer

                                                            link  Strep-tag®II
                                                          M  A  S  W  S  H  P

81  GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAGCTGGAGCCACCCGC   160
                                                 XbaI                  NheI

                                R  P  R  S  R  I  R  A  R  Y  P  G  I  P  R  G  R  P
                factor Xa      E  T  A  V  P  N  S  S  S  V  P  G  D  P  S  R  S  T  C
    Q  F  E  K  I  E  G  R  R  D  R  G  P  E  F  E  L  G  T  R  G  S  L  E  V  D  L

161 AGTTCGAAAAAATCGAAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTG   240
                      BbeI  BsaI      BsmFI     SstI  KpnI     BamHI       SalI  PstI
                      EheI    PshAI       EcoRI           SmaI       XhoI
                      KasI      SacII
                      NarI

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

241 CAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTT   320
        BsmFI   BsaI  EcoRV       HindIII
          PshAI
            NcoI

321 TGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTT   400
            reverse primer
```

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-IBA7C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag®II	139	171
Factor Xa cleavage site	172	183
multiple cloning site	184	260
reverse primer binding site	328	344
f1 origin	357	795
CamR resistance gene	917	1576
Tet-repressor	1589	2212
ColE1 origin	2365	2953



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