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Data Sheet

pASK-IBA5C

Cat. No.: 2-1324-000

Version: 11.0
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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.
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

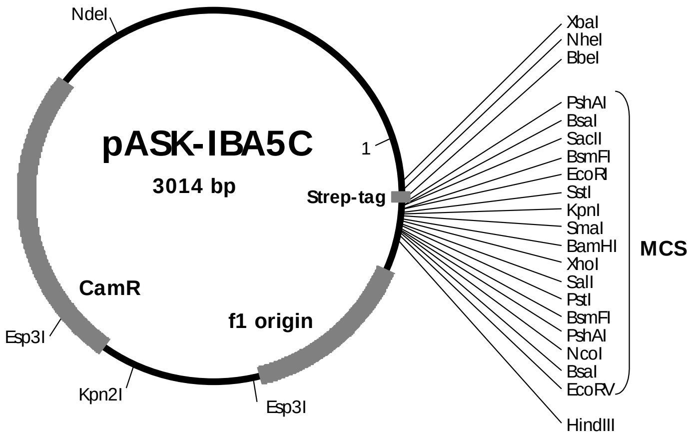
Multiple Cloning Site of pASK-IBA5C

```
1        CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA    80
                                                                   forward primer

                                                                      link    Strep-tag®II
                                                                   M  A S  W  S  H  P
81       GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAGCTGGAGCCACCCGC    160
                                             XbaI                    NheI

                         D  R  G  P  E  F  E  L  G  T  R  G  S  L  E  V  D  L  Q  G
               link    R  P  R  S  R  I  R  A  R  Y  P  G  I  P  R  G  R  P  A  G  G
         Q  F  E  K  G  A  E  T  A  V  P  N  S  S  S  V  P  G  D  P  S  R  S  T  C  R  G
161      AGTTCGAAAAAGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGG    240
                   BbeI  BsaI     BsmFI     SstI  KpnI     BamHI      SalI  PstI  BsmFI
                   EheI   PshAI        EcoRI          SmaI       XhoI                PshAI
                   KasI     SacII
                   NarI

         D  H  G  L  *
           P  W  S  L  I  S  N  *
          T  M  V  S  D  I  *
241      ACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGC    320
          NcoI       EcoRV      HindIII
             BsaI

321      CGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA    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-IBA5C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag®II	139	171
multiple cloning site	172	253
reverse primer binding site	321	337
f1 origin	350	788
CamR resistance gene	910	1569
Tet-repressor	1582	2205
ColE1 origin	2358	2946



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