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Data Sheet

pASK-IBA5C

Cat. No.: 2-1324-000
Lot No.: 1324-

Version: 10.3
Revision Date: 27.02.2020

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-Tactin® affinity tag (Strep-tag®II) 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 *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.

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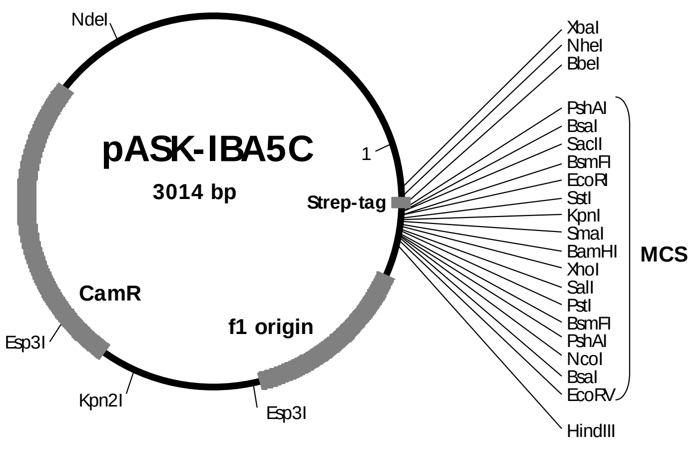
Multiple Cloning Site of pASK-IBA5C

```
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 e.g. for subcloning the recombinant gene into pEXPR-IBA vectors for mammalian expression.

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
Col E1origin	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'