Data Sheet

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pASK-IBA5C

Cat. no. : 2-1324-000

Lot no.: 1324-

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Version 1324-6

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
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

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. *Strep*-tag® and *Strep*-Tactin® are registered trademarks of IBA GmbH.

Multiple Cloning Site of pASK-IBA5C

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1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA   80
                                                                 forward primer

                                                                    link    Strep-tag
                                                                 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  *

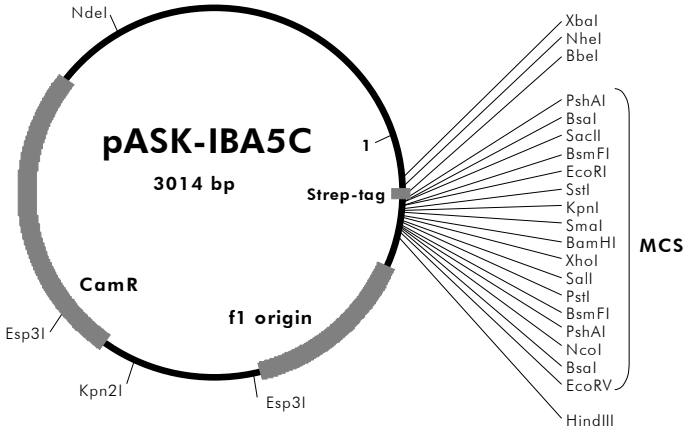
241     ACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGC   320
         NcoI       EcoRV      HindIII
             BsaI

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	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



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'