

## Data Sheet

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## pASK-IBA5C

Cat. No. : 2-1324-000

Lot No.: 1324-

Last date of revision  
May 10

Version 1324-9

<b>Description</b>	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm.
<b>Affinity tag</b>	<i>Strep-Tactin</i> <sup>®</sup> affinity tag ( <i>Strep-tag II</i> <sup>®</sup> ) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein.
<b>Bacterial Expression</b>	Expression is induced upon addition of 200 µg anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter <i>E. coli</i> shaking culture ( $A_{550} = 0.5$ ).
<b>Expression strain</b>	Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> .
<b>Resistance</b>	Chloramphenicol <b>Note:</b> The CamR resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of <i>E. coli</i> transformed with this plasmid
<b>Form</b>	5 µg, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 µl
<b>Concentration</b>	250 ng/µl
<b>Storage</b>	4 °C for frequent usage, -20 °C for long-term storage

## For research use only

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## Multiple Cloning Site of pASK-IBA5C

```

1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA  80
                                     forward primer

                                     link   Strep-tag
                                     M A S W S H P

81     GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAATCTAGATAACGAGGGCAAAAATGGCTAGCTGGAGCCACCCGC  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    AGTTCGAAAAAGgcgCGAGACCGGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTGCAGCTGCAGGGGG  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 *

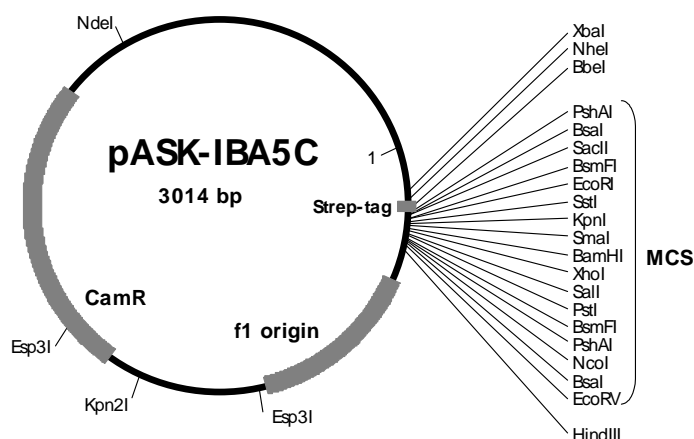
241    ACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTGTCTGC  320
      NcoI   EcoRV   HindIII
      BsaI

321    CGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGGCGGGTGTGGTGGTTACGCGCA  400
      reverse primer
  
```

Please note: Restriction enzymes in bold cut twice. The *BsaI* sites (isoschizomer of *Eco31I*) 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
Col E1 origin	2358	2946



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC <sup>(N<sub>20</sub>)</sup> NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA <sup>(N<sub>20</sub>)</sup> NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'