

## Data Sheet

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## pASK-IBA2C

Cat. No. : 2-1321-000

Lot No.: 1321-

Last date of revision  
May 10

Version 1321-9

<b>Description</b>	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be secreted into the periplasm.
<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 C-terminus of the recombinant protein.
<b>Secretion</b>	The ompA signal sequence directs the expressed protein into the periplasmic space and will be cleaved off during the translocation process
<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-IBA2C

```

1      CCATCGAATGGCCAGATGATTAATTCCTAAATTTTGTGGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA 80
                                     forward primer

                                     OmpA
                                     M K K T A I A
81      GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAAGACAGCTATCGCGA 160
                                     XbaI
OmpA
I A V A L A G F A T V A Q A G D H G P E F E L G T R G
161     TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAgcccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA 240
                                     BsaI   BsmFI   SstI   KpnI   BamHI
                                     PshAI   EcoRI   SmaI
                                     NcoI

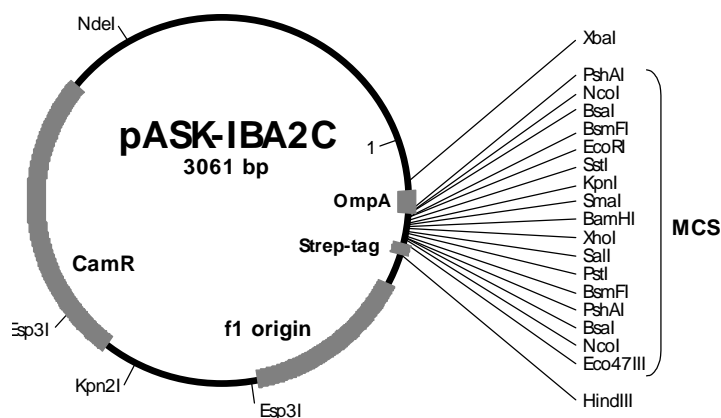
link      Strep-tag
S L E V D L Q G D H G L S A W S H P Q F E K *
241     TCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCagcgcTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGACC 320
XhoI SalI PstI BsmFI BsaI Eco47III HindIII
PshAI
NcoI

321     TGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGC 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. During secretion of the recombinant protein into the periplasmic space, the OmpA signal sequence will be cleaved off. The processed protein will start with the first amino acid after the last Alanine of the signal sequence.

## Features of pASK-IBA2C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
multiple cloning site	202	282
Strep-tag	283	312
reverse primer binding site	368	384
f1 origin	397	835
CamR resistance gene	957	1616
Tet-repressor	1629	2252
Col E1 origin	2405	2993



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