

## Data Sheet

# pASK-IBA16

Cat. No. : 2-1315-000

Lot No.: 1315 -

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<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</i> -Tactin affinity tag ( <i>Strep</i> -tag II) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein and can be removed by cleavage with TEV protease (tobacco etch virus). TEV protease is a site-specific protease with a seven amino acid recognition site (in pASK-IBA16: ENLYFQG) and cleavage occurs between glutamine (Q) and glycine (G).
<b>Bacterial Expression</b>	Expression is induced upon addition of 200 $\mu$ g 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>	Ampicillin
<b>Form</b>	5 $\mu$ g, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 $\mu$ l
<b>Concentration</b>	250 ng/ $\mu$ l
<b>Storage</b>	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-IBA16

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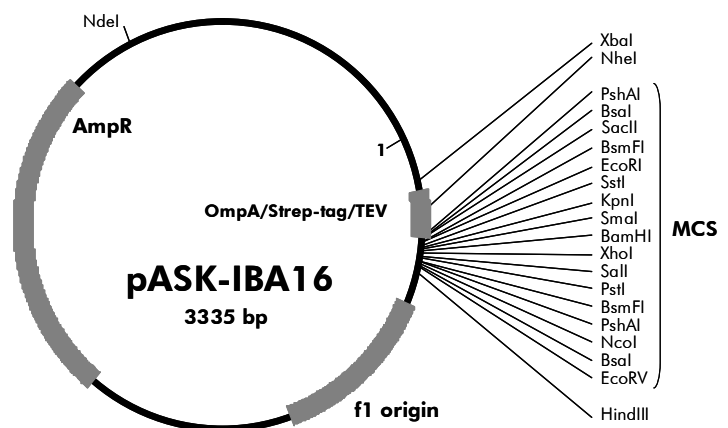
1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA      80
                                           forward primer
                                           M K K T A I A
81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAGACAGCTATCGCGA      160
                                           XbaI
                                           OmpA          link          Strep-tag          linker
I A V A L A G F A T V A Q A A S W S H P Q F E K S G G
161     TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGTAGCTGGAGCCACCCGAGTTCGAAAAATCTGGTGGT      240
                                           NheI
                                           D R G P E F E L G T R G S L E
TEV protease          R P R S R I R A R Y P G I P R
G G G E N L Y F Q G A E T A V P N S S S V P G D P S R
241     GGTGGTGGTGAGAATCTTTATTTTCAGGgcgCGAGACCGCGTCCCGAATTCGAGCTCGGTACCCGGGATCCCTCGAG      320
                                           BbeI   BsaI   BsmFI   SstI   KpnI   BamHI
                                           EheI   PshAI   EcoRI   SmaI   XhoI
                                           KasI   SacII
                                           NarI
                                           V D L Q G D H G L *
                                           G R P A G G P W S L I S N *
                                           S T C R G T M V S D I *
321     GTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTAAAAATGGCGCACATTGTGCGA      400
                                           SalI   PstI   BsmFI   BsaI   EcoRV   HindIII
                                           PshAI
                                           NcoI
401     CATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGGGT      480
                                           reverse primer

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**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 Ala-Ser-linker.

### Features of pASK-IBA16

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
TEV cleavage site	232	272
multiple cloning site	273	349
reverse primer binding site	417	433
f1 origin	446	884
AmpR resistance gene	1033	1893
tet-repressor	1903	2526



#### Cloning primers for the precise cloning using *BsaI* or *Eco31I*

Forward: 5'- NNNNNNGGTCTCNGC GCC <sup>(N<sub>20</sub>)</sup> NNN NNN...  
Reverse: 5'- NNNNNNGGTCTCNTA TCA <sup>(N<sub>20</sub>)</sup> NNN NNN...

#### Sequencing primers:

Forward: 5'- GAGTTATTTTACCACTCCCT -3'  
Reverse: 5'- CGCAGTAGCGGTAAACG -3'