

## Data Sheet

# pASK-IBA17plus

Cat. No. : 2-1416-000

Lot No.: 1416 -

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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 localized in the cytoplasm.
<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-IBA17plus: ENLYFQG) and cleavage occurs between glutamine (Q) and glycine (G).
<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>	Ampicillin
<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

*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-IBA17plus

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1          CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTGAC      41

42  ACTCTATCATTGATAGAGTTATTTTACCCTCCCTATCAGTGATAGAGAAAAGTAAATGAATAGTTCGACAAAAATCTA  121
      forward primer                                         XbaI

                                     link      Streptag      linker
                                     M A S W S H P Q F E K S G G

122  GAAATAATTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCTGGAGCCACCCGAGTTCGAAAAATCTGGTGGT  201
      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
202  GGTGGTGGTGAGAATCTTTATTTTCAGGgcgCGAGACCGGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAG  281
      BbeI  BsaI  BsmFI  SstI  KpnI  BamHI
      EheI  PshAI  EcoRI  SmaI  XhoI
      KasI  SacII
      NarI

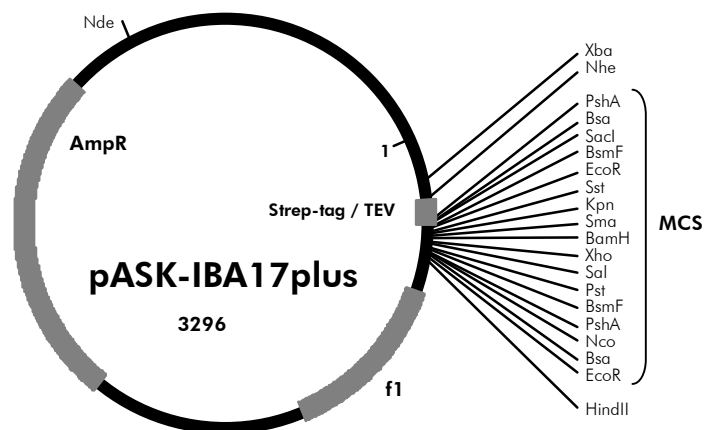
      V D L Q G D H G L *
      G R P A G G P W S L I S N *
      S T C R G G T M V S D I *
282  GTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTAAAAATGGCGCACATTGTGCGA  361
      SalI  PstI  BsmFI  BsaI  EcoRV  HindIII
      PshAI
      NcoI

362  CATTTTTTTGTCTGCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGGGCGGGT  441
      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-IBA17plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
TEV cleavage site	211	231
multiple cloning site	232	318
reverse primer binding site	378	394
f1 origin	407	845
AmpR resistance gene	994	1854
Tet-repressor	1864	2489



#### 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'- GAGTTATTTTACCCTCCCT -3'  
 Reverse: 5'- CGCAGTAGCGGTAACG -3'