Data Sheet

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Version 1416-3

pASK-IBA17plus

Cat. No. : 2-1416-000

Lot No.: 1416 -

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 and can be removed by cleavage with TEV protease (tabacco 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).
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	Ampicillin
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

Multiple Cloning Site of pASK-IBA17plus

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1                                                 CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGAC    41

42     ACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTA   121
                      forward primer                                                  XbaI

                                                link       Streptag          linker
                                              M  A  S  W  S  H  P  Q  F  E  K  S  G  G
122    GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCTGGTGGT   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    GGTGGTGGTGAGAATCTTTATTTTCAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAG   281
                                   BbeI  BsaI    BsmFI    SstI  KpnI    BamHI
                                   EheI   PshAI        EcoRI         SmaI      XhoI
                                   KasI     SacII
                                   NarI

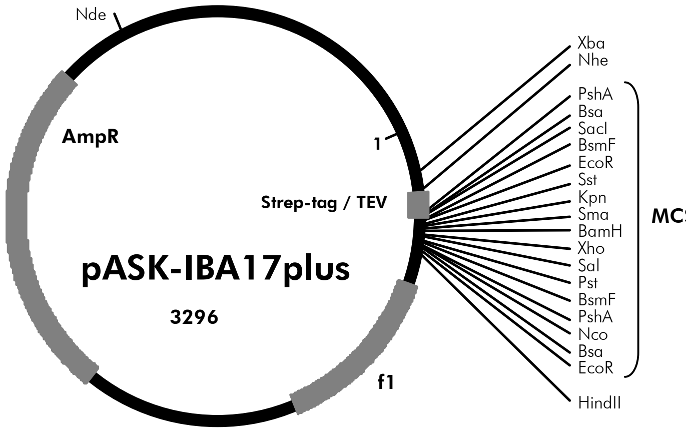
        V  D  L  Q  G  D  H  G  L  *
       G  R  P  A  G  G  P  W  S  L  I  S  N  *
         S  T  C  R  G  T  M  V  S  D  I  *
282    GTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGA   361
       SalI  PstI  BsmFI  BsaI  EcoRV   HindIII
                     PshAI
          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

362    CATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGT   441
                        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-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 *Bsa*I or *Eco31*I	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC NNN NNN... (N_{20})	Forward:5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA NNN NNN... (N_{20})	Reverse:5'- CGCAGTAGCGGTAAACG -3'