



Data Sheet

pASK-IBA5plus

Cat. no. : 2-1404-000

Lot no.: 1404-

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Version 1404-6

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	<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.
Bacterial Expression	Expression is induced upon addition of 200 μ g anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter <i>E. coli</i> shaking culture ($A_{550} = 0.5$).
Expression strain	Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> .
Resistance	Ampicillin
Form	5 μ g, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 μ l
Concentration	250 ng/ μ l
Storage	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-IBA5plus

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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC 79
                                     forward primer

80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAAATAATTTTTGTTTAACTTTAAGAAGGAGATATACAA 159
                                     XbaI

                                     D R G P E F E L G T R G S
Strep-tag      link      R P R S R I R A R Y P G I
M A S W S H P Q F E K G A E T A V P N S S S V P G D P

160    ATGGCTAGCTGGAGCCACCCGCGAGTTTCGAAAAAGgcgCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCC 239
      NheI      BbeI  BsaI  BsmFI  SstI  KpnI  BamHI
                                     EheI  PshAI  EcoRI      SmaI      XhoI
                                     KasI  SacII
                                     NarI

      L E V D L Q G D H G L *
      P R G R P A G G P W S L I S N *
      S R S T C R G T M V S D I *

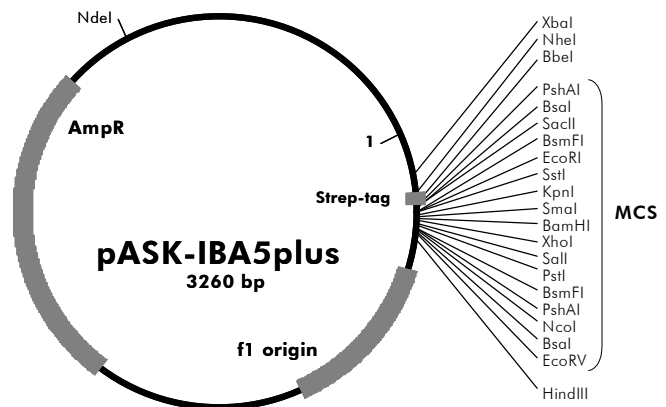
240    CTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTAAAAATGGCGCACATT 319
      SalI  PstI  BsmFI  BsaI  EcoRV      HindIII
                                     PshAI
                                     NcoI

320    GTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCG 399
                                     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.

Features of pASK-IBA5plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
multiple cloning site	193	274
reverse primer binding site	342	358
f1 origin	371	809
AmpR resistance gene	958	1818
Tet-repressor	1828	2451



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

Forward: 5'- NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN...
 Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN...

Sequencing primers:

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