

Data Sheet

pASK-IBA45plus

Cat. no. : 2-1445-000

Lot no.: 1445-

IBA Headquarters

IBA GmbH
Rudolf-Wissell-Str. 28
D-37079 Göttingen
Germany
Tel. +49 (0) 551-5 06 72-0
Fax +49 (0) 551-5 06 72-181
E-mail info@iba-go.com
<http://www.iba-go.com>

IBA US Distribution Center

10748 Indianhead Industrial Blvd.
St. Louis, MO 63132
USA
Tel. 1-877-IBA-GmbH
(1-877-422-4624)
Fax 1-888-531-6813
E-mail info@iba-go.com
<http://www.iba-go.com>

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Version 1445-7

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	The recombinant protein will contain two affinity tags: 1) <i>Strep</i> -Tactin affinity tag (<i>Strep</i> -tag II) for the purification of recombinant protein via <i>Strep</i> -Tactin resins. The <i>Strep</i> -tag is fused to the N-terminus of the recombinant protein. 2) 6xHistidine-tag for the purification of recombinant protein via Ni-NTA resins. The 6xHistidine-tag is fused to the C-terminus of the recombinant protein.
Bacterial Expression	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 ($A_{550} = 0.5$).
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 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

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. The 6xHistidine-tag is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche Ltd., Basel, Switzerland and is provided only for the use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

Multiple Cloning Site of pASK-IBA45plus

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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC 79
      forward primer

80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACAA 159
      XbaI

      link      Strep-tag      link
      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    ATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGggcgcCGAGACCGCGTCCCGAATTCGAGCTCGGTACCCGGGGATCC 239
      NheI      BbeI BsaI BsmFI SstI KpnI BamHI
      EbeI PshAI EcoRI SmaI
      KasI SacII
      NarI

      6xHistidine-tag
      S R S T C R G T M V S G L R G S H H H H H H *

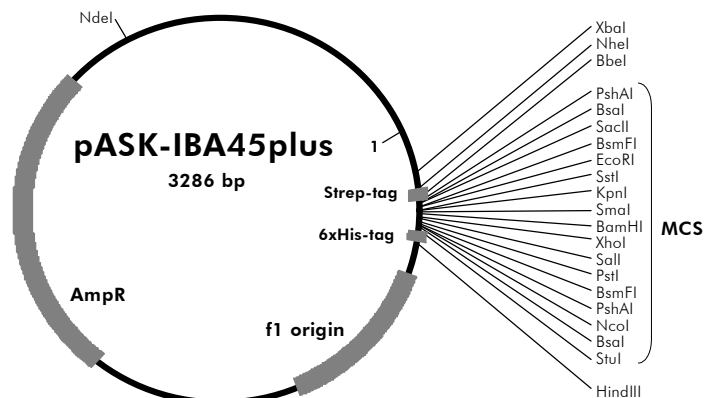
240    CTCGAGGTCGACCTGCAGGGGACCATGGTCTCaggccTGAGAGGATCGCATCACCATCACCATCACTAATAAGCTTGAC 319
      XhoI SalI PstI BsmFI BsaI StuI HindIII
      PshAI
      NcoI

320    CTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACG 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-IBA45plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
multiple cloning site	193	279
6xHistidine-tag	280	309
reverse primer binding site	368	384
f1 origin	397	835
AmpR resistance gene	984	1844
Tet-repressor	1854	2477



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

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

Sequencing primers:

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