

Data Sheet

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pASK-IBA37plus

Cat. no. : 2-1437-000

Lot no.: 1437-

Last date of revision
July 05

Version 1437-7

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	6xHistidine-tag 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 factor Xa.
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-LaRoche 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-IBA37plus

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1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC 79
                                     forward primer

80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAATAATTTTTGTTTAACTTTAAGAAGGAGATATACAA 159
                                     XbaI
          link      6xHistidine-tag      factor Xa      R P R S R I R A R Y
          M A S R G S H H H H H H I E G R E T A V P N S S S V
160    ATGGCTAGCAGAGGATCGCATCACCATCACCATCACATCGAAGGgcgCGAGACCGGGTCCCGAATTCGAGCTCGGTAC 239
          NheI                                     BbeI BsaI BsmFI SstI KpnI
          EheI PshAI EcoRI SmaI
          KasI SacII
          NarI

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

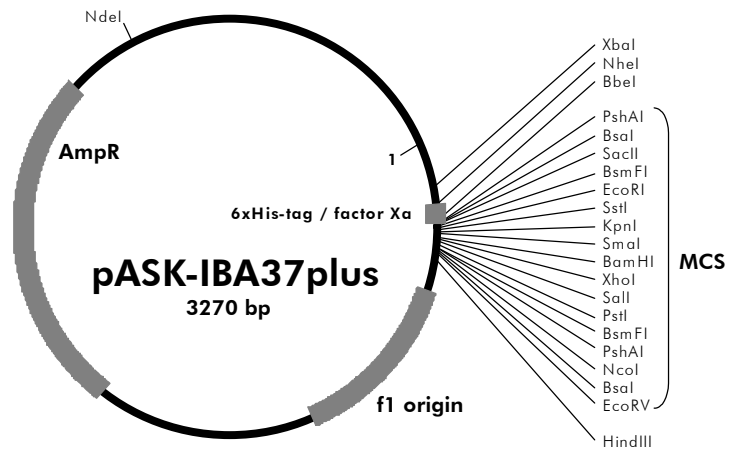
240    CCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataCTAACTAAGCTTGACCTGTGAAGTAAAAAT 319
          BamHI SalI PstI BsmFI BsaI EcoRV HindIII
          XhoI PshAI
          NcoI

320    GGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGC 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-IBA37plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
6xHistidine-tag	160	195
factor Xa cleavage site	196	207
multiple cloning site	208	284
reverse primer binding site	352	368
f1 origin	381	819
AmpR resistance gene	968	1828
tet-repressor	1838	2461



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

Forward: 5'- NNNNNNGGTCTCNG CGC ^(N₂₀) NNN NNN...
 Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN...

Sequencing primers:

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