

## Data Sheet

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# pASK-IBA35plus

Cat. no. : 2-1435-000

Lot no.: 1435-

Last date of revision  
**July 05**

Version 1435-7

<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>	6xHistidine-tag for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein.
<b>Bacterial Expression</b>	Expression is induced upon addition of 200 $\mu$ g 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 $\mu$ g, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 $\mu$ l
<b>Concentration</b>	250 ng/ $\mu$ l
<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. 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-IBA35plus

1 CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTGACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC 79  
forward primer

80 AGTGATAGAGAAAAGTGAATAGTAGTTCGACAAAAATCTAGAATAATTTTTGTTAACTTTAAGAAGGAGATATACAA 159  
XbaI

link 6xHistidine-tag link D R G P E F E L G T R G  
M A S R G S H H H H H G A E T A V P N S S S V P G D

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

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

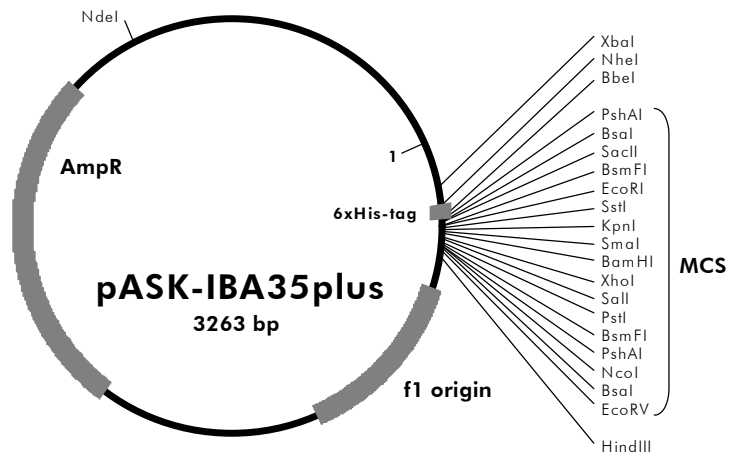
240 TCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGA AAAATGGCGCAC 319  
XhoI SalI PstI **BsmFI** **BsaI** EcoRV HindIII  
**PshAI**  
NcoI

320 ATGTGCGACATTTTTTTGTCTGCCGTTTACCCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGC 399  
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-IBA35plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
6xHistidine-tag	160	195
multiple cloning site	196	277
reverse primer binding site	345	361
f1 origin	374	812
AmpR resistance gene	932	1792
Tet-repressor	1802	2425



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