

## Data Sheet

# pASK-IBA15plus

Cat. No. : 2-1414-000

Lot No.: 1414-

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Last date of revision

July 05

Version 1414-6

<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>	<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 and can be removed by cleavage with Enterokinase.
<b>Bacterial Expression</b>	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 ( $A_{550} = 0.5$ ).
<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 µg, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 µl
<b>Concentration</b>	250 ng/µl
<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.

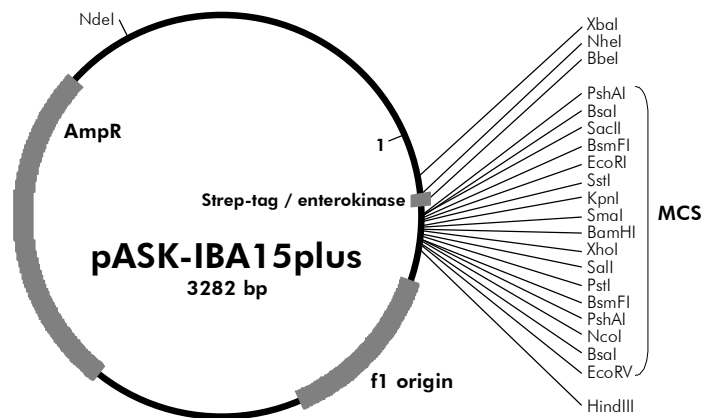
## Multiple Cloning Site of pASK-IBA15plus

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC	80
	forward primer	
81	AGTGATAGAGAAAAGTGAATAGTTCGACAAAAATCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACAA	160
	XbaI	
	<div style="display: flex; justify-content: space-around; font-size: small;"> <span>link</span> <span>Strep-tag</span> <span>enterokinase</span> <span>P R P R S R I</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>M A S W S H P Q F E K G A D D D D K G S R D R G P E F</span> <span>P E T A V P N</span> </div>	
161	ATGGCTAGCTGGAGCCACCCGAGTTCGAAAAAGGCGCCGACGACGACACAAGGGctccCGAGACCCGGTCCCGAATT	240
	<div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>NheI</span> <span>BbeI</span> <span>BsaI</span> <span>BsmFI</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>EheI</span> <span>EcoRI</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>KasI</span> <span>SacII</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>NarI</span> </div>	
	<div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>R A R Y P G I P R G R P A G G P W S L I S N *</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>S S S V P G D P S R S T C R G T M V S D I *</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>E L G T R G S L E V D L Q G D H G L *</span> </div>	
241	CGAGCTCGGTACCCGGGATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGT	320
	<div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>SstI</span> <span>KpnI</span> <span>BamHI</span> <span>SalI</span> <span>PstI</span> <span>BsmFI</span> <span>BsaI</span> <span>EcoRV</span> <span>HindIII</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>SmaI</span> <span>XhoI</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>PshAI</span> </div> <div style="display: flex; justify-content: space-around; font-size: x-small;"> <span>NcoI</span> </div>	
321	GAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTTTGTCTGCCGTTTACCGCTACTGCGTACCGGATCTCCACGCGCCC	400
	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-IBA15plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
enterokinase cleavage site	193	213
multiple cloning site	214	296
reverse primer binding site	364	380
f1 origin	393	831
AmpR resistance gene	980	1840
Tet-repressor	1850	2473



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNC TCC NNN NNN... <sup>(N<sub>20</sub>)</sup>	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA NNN NNN... <sup>(N<sub>20</sub>)</sup>	Reverse: 5'- CGCAGTAGCGGTAACG -3'