

## Data Sheet

# pASK-IBA2

Cat. no. : 2-1301-000

Lot no.: 1301-

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<b>Description</b>	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be secreted into the periplasm.
<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 C-terminus of the recombinant protein.
<b>Secretion</b>	The ompA signal sequence directs the expressed protein into the periplasmic space and will be cleaved off during the translocation process
<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.

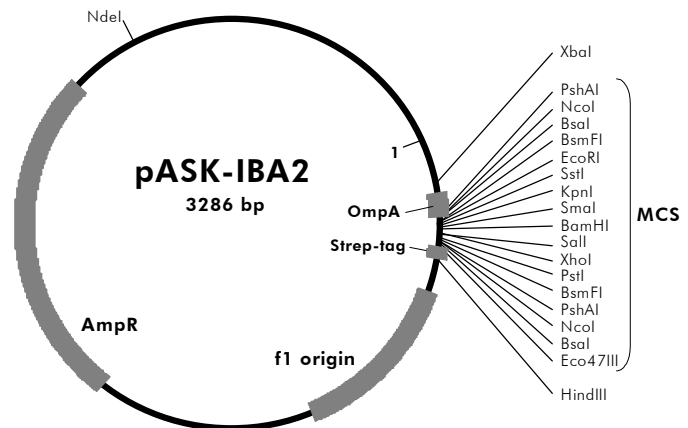
## Multiple Cloning Site of pASK-IBA2

1	CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA	80
	forward primer	
	M K K T A I A	
81	GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAGACAGCTATCGCGA	160
	XbaI	
	OmpA	
	I A V A L A G F A T V A Q A G D H G P E F E L G T R G	
161	TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAggccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA	240
	BsaI BsmFI SstI KpnI BamHI PshAI EcoRI SmaI NcoI	
	link Strep-tag	
	S L E V D L Q G D H G L S A W S H P Q F E K *	
241	TCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCAGcgctTTGGAGCCACCCGAGTTCGAAAAATAAAGCTTGACC	320
	XhoI SalI PstI BsmFI BsaI Eco47III HindIII PshAI NcoI	
321	TGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGC	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. During secretion of the recombinant protein into the periplasmic space, the OmpA signal sequence will be cleaved off. The processed protein will start with the first amino acid after the last Alanine of the signal sequence.

## Features of pASK-IBA2

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
multiple cloning site	202	282
Strep-tag	283	312
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 <sup>(N<sub>20</sub>)</sup> NNN NNN...  
 Reverse: 5'- NNNNNNGGTCTCNGC GCT <sup>(N<sub>20</sub>)</sup> NNN NNN...

### Sequencing primers:

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