

## Data Sheet

# pASK-IBA12

Cat. no. : 2-1311-000

Lot no.: 1311 -

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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 N-terminus of the recombinant protein and can be removed by cleavage with Thrombin. The cleavage is enhanced due to a "kinker" site <sup>1)</sup> .
<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

1) Hakes, J.D. & Dixon, J.E. (1992): *Anal. Biochem.* 202, 293-298.

## 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-IBA12

1 CCATCGAATGGCCAGATGATTAATTCCTAAATTTTGTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA 80  
 forward primer  
 M K K T A I A

81 GTGATAGAGAAAAGTGAATGAATAGTTCGACAAAATCTAGATAACGAGGGCAAAAATGAAAAGACAGCTATCGCGA 160  
 XbaI

I A V A L A G F A T V A Q A A S W S H P Q F E K S G G  
 OmpA link Strep-tag kinker

161 TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGAGTTCGAAAAATCTGGTGGT 240  
 NheI

R P R S R I R A R Y P G I P R G R  
 thrombin E T A V P N S S S V P G D P S R S  
 G G G L V P R G S R D R G P E F E L G T R G S L E V D

241 GGTGGTGGTCTGGTTCCGCGTGGGtccCGAGACCGCGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGA 320  
 BsaI BsmFI SstI KpnI BamHI SalI  
 PshAI EcoRI SmaI XhoI  
 SacII

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

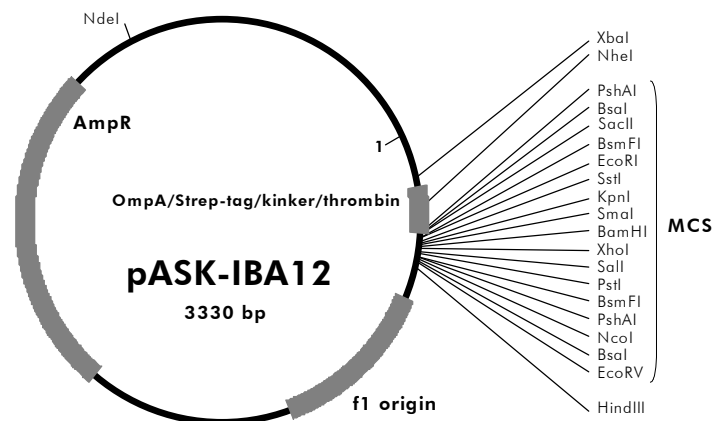
321 CCTGCAGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTT 400  
 PstI BsmFI BsaI EcoRV HindIII  
 PshAI  
 NcoI

401 TTTTGTCTGCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGGGGGTGTGGT 480  
 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. 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 Ala-Ser-linker.

## Features of pASK-IBA12

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
kinker	232	249
thrombin cleavage site	250	267
multiple cloning site	268	344
reverse primer binding site	412	428
f1 origin	441	879
AmpR resistance gene	1028	1888
tet-repressor	1898	2521



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

Forward: 5'- NNNNNNGGTCTCNC TCC <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'