

Data Sheet

IBA Headquarters
IBA GmbH
Rudolf-Wissell-Str. 28
D-37079 Göttingen
Germany
Tel. +49 (0) 551-5 06 72-0
Fax +49 (0) 551-5 06 72-181
E-mail info@iba-go.com
<http://www.iba-go.com>

IBA US Distribution Center
10748 Indianhead Industrial Blvd.
St. Louis, MO 63132
USA
Tel. 1-877-IBA-GmbH
(1-877-422-4624)
Fax 1-888-531-6813
E-mail info@iba-go.com
<http://www.iba-go.com>

pASK-IBA14

Cat. no. : 2-1313-000

Lot no.: 1313 -

Last date of revision
July 05

Version 1313-6

Description	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be secreted into the periplasm.
Affinity tag	<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.
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.

Multiple Cloning Site of pASK-IBA14

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

81 GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAGACAGCTATCGCGA 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 G A D

161 TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGAGTTCGAAAAAGGCCCGCAG 240
 link Strep-tag
 OmpA
 I A V A L A G F A T V A Q A A S W S H P Q F E K G A D
 enterokinase A P E T A V P N S S S V P G D P S R S T C R
 D D D K G S R D R G P E F E L G T R G S L E V D L Q G

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

G P W S L I S N *
 G T M V S D I *
 D H G L *

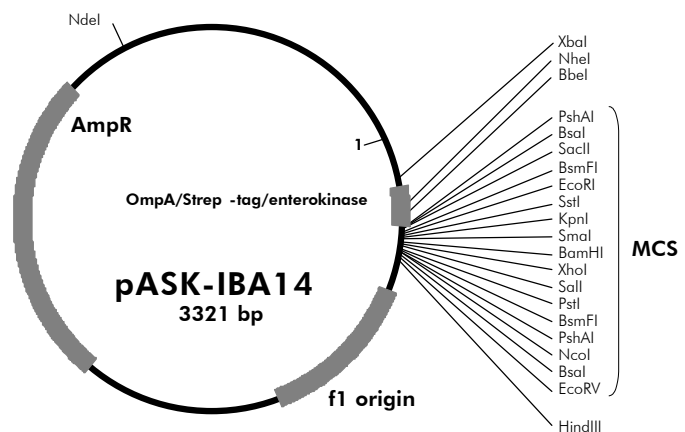
321 GGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTAAAAATGGCGCACATTGTGCGACATTTTTTTGTCT 400
 PshAI BsaI EcoRV HindIII
 NcoI

401 GCCGTTTACCGCTACTGCGTACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGGGCGGGTGTGGTGGTTACGCG 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-IBA14

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag	202	231
enterokinase cleavage site	232	252
multiple cloning site	253	335
reverse primer binding site	403	419
f1 origin	432	870
AmpR resistance gene	1019	1879
tet-repressor	1889	2512



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

Forward: 5'- NNNNNNGGTCTCNC TCC NNN NNN...^(N₂₀)

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

Sequencing primers:

Forward: 5'- GAGTTATTTTACCACTCCCT -3'

Reverse: 5'- CGCAGTAGCGGTAACG -3'