

Data Sheet

pASK-IBA32

Cat. no. : 2-1332-000

Lot no.: 1332 -

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Version 1332-9

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	6xHistidine-tag for the purification of recombinant protein. The affinity tag is fused to the C-terminus of the recombinant protein.
Secretion	The ompA signal sequence directs the expressed protein into the periplasmic space and will be cleaved off during the translocation process
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. The 6xHistidine-tag is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche 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.

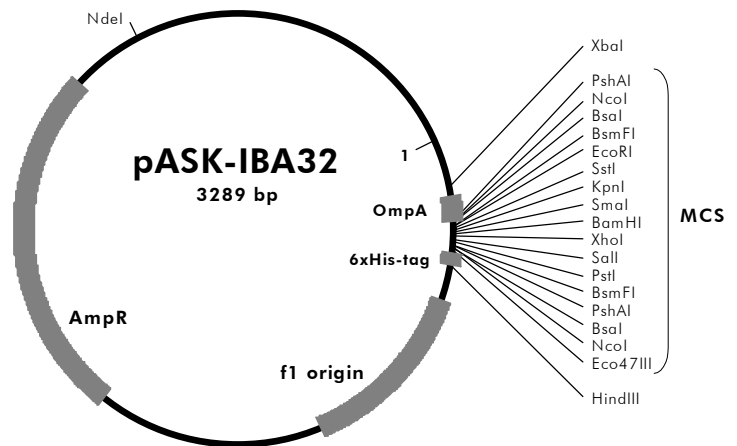
Multiple Cloning Site of pASK-IBA32

1	CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA	80
	forward primer	
	M K K T A I A	
81	GTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGAAAAGACAGCTATCGCGA	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	TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAggcGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA	240
	BsaI BsmFI SstI KpnI BamHI PshAI EcoRI SmaI NcoI	
	link 6xHistidine-tag	
	S L E V D L Q G D H G L S A R G S H H H H H H *	
241	TCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCagcgcTAGAGGATCGCATCACCATCACCATCAATAAGCTTG	320
	XhoI SalI PstI BsmFI BsaI Eco47III HindIII PshAI NcoI	
321	ACCTGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTGTCTGCCGTTTACCGTACTGCGTCACGGATCTCCA	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. 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-IBA32

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
multiple cloning site	202	282
6xHistidine-tag	283	312
reverse primer binding site	371	387
f1 origin	400	838
AmpR resistance gene	987	1847
tet-repressor	1857	2480



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

Forward: 5'- NNNNNNGGTCTCNG GCC ^(N₂₀) NNN NNN...
 Reverse: 5'- NNNNNNGGTCTCNGC GCT ^(N₂₀) NNN NNN...

Sequencing primers:

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