

# **Data Sheet**

## pASK-IBA3C

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Cat. No.: Lot No.:	2-1322-00 1322-	0 Last date of revision March 2012 Version 1322-9
Description		Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm.

	the cytoplasm.	
Affinity tag	<i>Strep</i> -Tactin <sup>®</sup> affinity tag ( <i>Strep</i> -tag II <sup>®</sup> ) for the purification of recombinant protein. The affinity tag is fused to the C-terminus of the recombinant protein.	
Bacterial Expression	Expression is induced upon addition of 200 $\mu$ g anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter <i>E. coli</i> shaking culture (A <sub>550</sub> = 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	Chloramphenicol <b>Note:</b> The CamR resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of E.coli transformed with this plasmid	
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

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### Multiple Cloning Site of pASK-IBA3C

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA	80
81	M G D R G P E GTGATAGAGAAAAGTGAAATGAAAAGTCGACAAAAAATCTAGATAACGAGGGCAAAAaatgGGAGACCGCGGTCCCGAAT XbaI BsaI BsmFI PshAI EcoRI SacII	160
161	link Strep-tag F E L G T R G S L E V D L Q G D H G L <u>S A W S H P Q F</u> <b>TCGAGCTCGGGACCCTGCAGGGGGCCCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCGCAGTTC</b> SstI KpnI BamHI Sall PstI <b>BsmFI BsaI</b> Eco47III SmaI XhoI <b>PshAI</b> NcoI E K *	240
241	GAAAAATAATAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTGTCTGCCGCTTACCGCTAC HindIII reverse primer	320
321	$\underline{\texttt{TGCG}} \texttt{TCACGGATCTCCACGCGCCCTGTAGCGGCGCCATTAAGCGCGGCGGGTGTGGTGGTGGTACGCGCAGCGTGACCGCTAC}$	400

**Please note:** Restriction enzymes in bold cut twice. The *Bsa*I sites (isoschizomer of *Eco31*I) 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-IBA3C

			Ndel
	from bp	to bp	PshAl Bsal
promoter	37	72	- SacIl BsmFI
forward primer binding site	57	76	
multiple cloning site	139	222	pASK-IBA3C 1 3001 bp
Strep-tag	223	252	BamHI Xhol
reverse primer binding site	308	324	Strep-tag
f1 origin	337	775	- CamR BsmFl PshAl
CamR resistance gene	897	1556	Ncol Bsal
Tet-repressor	1569	2192	Esp3I f1 origin
Col E1origin	2345	2933	Kpn2l
	•	•	Esp3l

Cloning prin	ners for the precise cloning using Bsal or Eco31	Sequencing primers:	
Forward:	$(N_{17})$ * 5'- NNNNNGGTCTCNA ATG NNN NNN	Forward: 5'- GAGTTATTTTACCACTCCCT -3'	
Reverse:	(N <sub>20</sub> ) 5'- NNNNNGGTCTCNGC GCT NNN NNN	Reverse: 5'- CGCAGTAGCGGTAAACG -3'	

\* The ATG start codon is already included