

Data Sheet

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pASK-IBA2C

Cat. No. : 2-1321-000

Lot No.: 1321-

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Version 1321-9

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	<i>Strep-Tactin</i> [®] affinity tag (<i>Strep-tag II</i> [®]) 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	Chloramphenicol Note: The CamR resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of <i>E. coli</i> 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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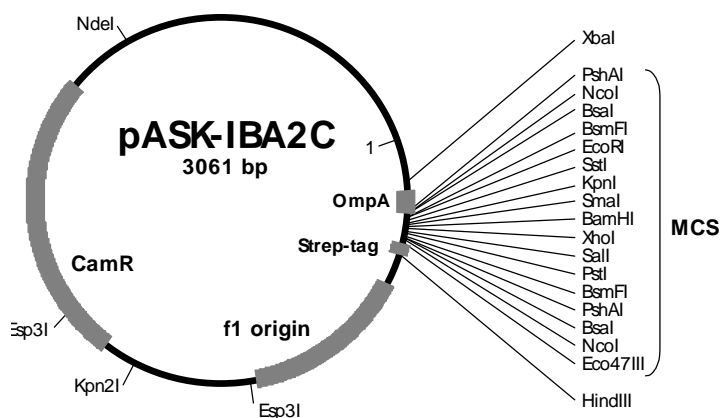
Multiple Cloning Site of pASK-IBA2C

1	CCATCGAATGGCCAGATGATTAATTCCTAAATTTTGTGGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA	80
	forward primer	
	OmpA M K K T A I A	
81	GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAAGACAGCTATCGCGA	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	TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAgcccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA	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	TCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCagcgcTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGACC	320
	XhoI SalI PstI BsmFI BsaI Eco47III HindIII PshAI NcoI	
321	TGTGAAGTGA AAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGTACTGCGTACCGGATCTCCACGC	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-IBA2C

	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
CamR resistance gene	957	1616
Tet-repressor	1629	2252
Col E1 origin	2405	2993



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNG GCC ^(N₂₀) NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT ^(N₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'