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Data Sheet

pASK-IBA2C

Cat. No.: 2-1321-000

Version: 12.0
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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	Strep-tag®II fused to the C-terminus of the recombinant protein.
Secretion	The <i>ompA</i> 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 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 Cam ^R 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 20 µl TE buffer, pH 8.0: 10 mM Tris/HCl, 1 mM EDTA
Concentration	250 ng/µl
Stability	12 months after shipping
Storage	recommended: 2-8 °C for frequent usage, -20 °C for long-term storage
Shipping	room temperature
Hazards	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

For research use only

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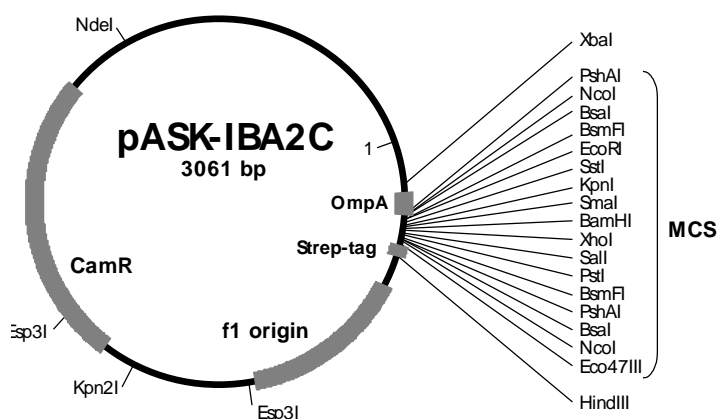
Multiple Cloning Site of pASK-IBA2C

1	CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA	80
	forward primer	
	M K K T A I A	
	OmpA	
81	GTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAACTAGATAACGAGGGCAAAAAATGAAAAGACAGCTATCGCGA	160
	XbaI	
	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	
	OmpA	
161	TTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGAagccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGA	240
	BsaI BsmFI SstI KpnI BamHI	
	PshAI EcoRI SmaI	
	NcoI	
	S L E V D L Q G D H G L S A W S H P Q F E K *	
	link Strep-tag [®] II	
241	TCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCagcgCTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGACC	320
	XhoI SalI PstI BsmFI BsaI Eco47III HindIII	
	PshAI	
	NcoI	
321	TGTGAAGTGAAAATGGCGCACATTGTGCGACATTTTTTTGTCTGCCGTTTACCGCTACTGCGTACCGGATCTCCACGC	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. 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 [®] II	283	312
reverse primer binding site	368	384
f1 origin	397	835
CamR resistance gene	957	1616
Tet-repressor	1629	2252
ColE1 origin	2405	2993



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNG GCC (N ₂₀) NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC (N ₂₀) GCT NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'