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Data Sheet

pASK-IBA6C

Cat. No.: 2-1325-000

Version: 11.0
Revision Date: 11.06.2021

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 N-terminus of the recombinant protein and can be removed by cleavage with Factor Xa.
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.

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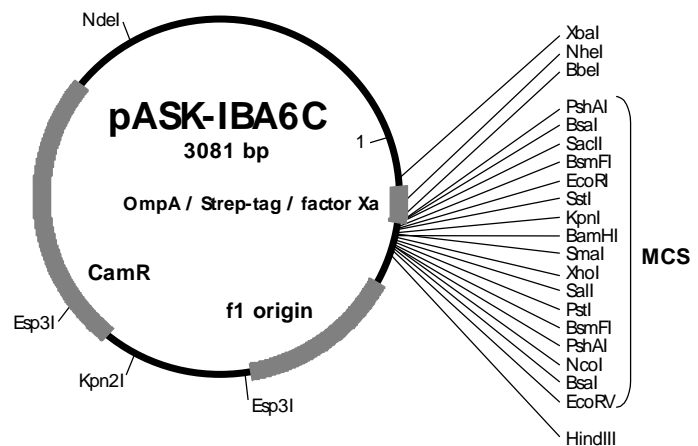
Multiple Cloning Site of pASK-IBA6C

1	CCATCGAATGGCCAGATGATTAATTCTAATTTTTGTTGACTCTATCATTGATAGAGTTATTTTACCCTCCCTAT forward primer	78
	M K K T A I A	
79	CAGTGATAGAGAAAAGTGAAATGAATAGTTTCGACAAAAATCTAGATAACGAGGGCAAAAATGAAAAGACAGCTATCGC XbaI	158
	OmpA link Strep-tag®II Factor Xa I A V A L A G F A T V A Q A A S W S H P Q F E K I E	
159	GATTGCAGTGGCAGCTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCGAAG NheI	238
	R P R S R I R A R Y P G I P R G R P A G G P W S E T A V P N S S S V P G D P S R S T C R G T M V S G R R D R G P E F E L G T R G S L E V D L Q G D H G L	
239	GgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTC BbeI BsaI BsmFI SstI KpnI BamHI SalI PstI BsmFI BsaI EheI PshAI EcoRI SmaI XhoI PshAI KasI SacII NcoI NarI	318
	L I S N * D I * *	
319	TgataCTAACTAAGCTTGACCTGTGAAGTGAATAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCCT EcoRV HindIII reverse primer	398
399	ACTGCGTCACGGATCTCCACGCGCCCTGTAGCGCGCATTAAAGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT	478

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 for subcloning.

Features of pASK-IBA6C

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
OmpA signal sequence	139	201
Strep-tag®II	202	231
Factor Xa cleavage site	232	243
multiple cloning site	244	320
reverse primer binding site	388	404
f1 origin	417	855
CamR resistance gene	977	1636
Tet-repressor	1649	2272
ColE1 origin	2425	3013



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNG CGC (N ₂₀) NNN NNN...	Forward: 5'- GAGTTATTTTACCCTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA (N ₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'