

Data Sheet

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pASK-IBA3plus

Cat. No. : 2-1402-000

Lot No.: 1402-

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May 10

Version 1402-9

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.
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.
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

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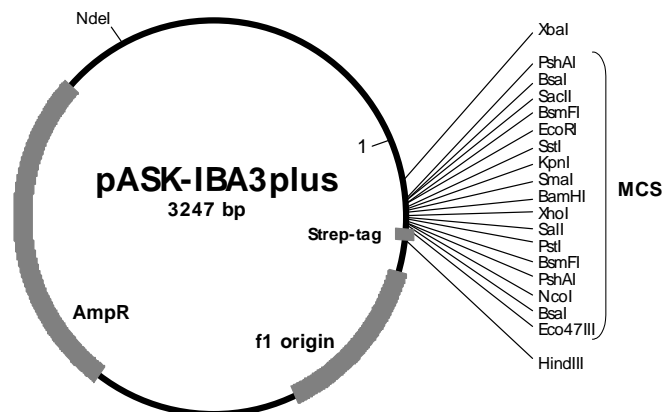
Multiple Cloning Site of pASK-IBA3plus

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACCTCCCTA	77
	forward primer	
78	TCAGTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATAC	157
	XbaI	
	M G D R G P E F E L G T R G S L E V D L Q G D H G L	
158	AaatGGGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTC	237
	BsaI BsmFI SstI KpnI BamHI SalI PstI BsmFI BsaI PshAI EcoRI SmaI XhoI PshAI SacII NcoI	
	link Strep-tag S A W S H P Q F E K *	
238	AgcgcTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATT	317
	Eco47III HindIII	
318	TTTTTGTCTGCCGTTTACCGCTACTGCGTACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGTGTGGT	397
	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.

Features of pASK-IBA3plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
multiple cloning site	160	243
Strep-tag	244	273
reverse primer binding site	329	345
f1 origin	358	796
AmpR resistance gene	945	1805
Tet-repressor	1815	2438
Col E1 origin	2591	3179



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNA ATG ^(N₁₇) * NNN NNN...	Forward: 5'- GAGTTATTTTACCACCTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT ^(N₂₀) NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'

* The ATG start codon is already included