

Data Sheet

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pASK-IBA5plus

Cat. No. : 2-1404-000

Lot No.: 1404-

Last date of revision
May 10

Version 1404-8

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 N-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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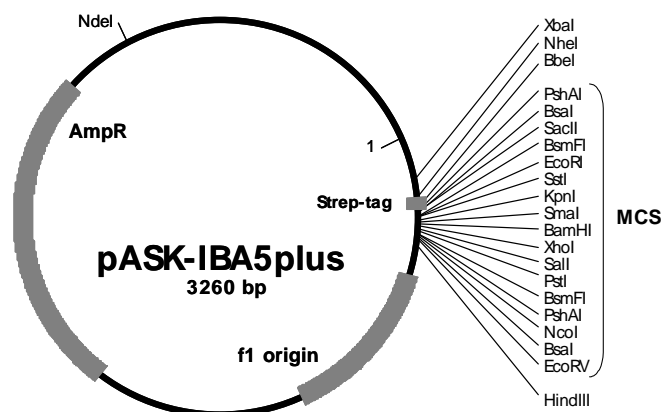
Multiple Cloning Site of pASK-IBA5plus

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTGTGGACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC	79
	forward primer	
80	AGTGATAGAGAAAAGTGAATGAATAGTTTCGACAAAAATCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACAA	159
	XbaI	
	D R G P E F E L G T R G S link R P R S R I R A R Y P G I Strep-tag M A S W S H P Q F E K G A E T A V P N S S S V P G D P	
160	ATGGCTAGCTGGAGCCACCCGAGTTCGAAAAAGgcgCGAGACCCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCC	239
	NheI BbeI BsaI BsmFI SstI KpnI BamHI EheI PshAI EcoRI SmaI XhoI KasI SacII NarI	
	L E V D L Q G D H G L * P R G R P A G G P W S L I S N * S R S T C R G T M V S D I *	
240	CTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATT	319
	SalI PstI BsmFI BsaI EcoRV HindIII PshAI NcoI	
320	GTGCGACATTTTTTTTGTCTGCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCG	399
	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.

Features of pASK-IBA5plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
multiple cloning site	193	274
reverse primer binding site	342	358
f1 origin	371	809
AmpR resistance gene	958	1818
Tet-repressor	1828	2451
Col E1 origin	2604	3192



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