

Data Sheet

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pASK-IBA45plus

Cat. No. : 2-1445-000

Lot No.: 1445-

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Version 1445-9

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	The recombinant protein will contain two affinity tags: <ol style="list-style-type: none"> 1) <i>Strep-Tactin</i>[®] affinity tag (<i>Strep-tag II</i>[®]) for the purification of recombinant protein via <i>Strep-Tactin</i> resins. The <i>Strep-tag</i> is fused to the N-terminus of the recombinant protein. 2) 6xHistidine-tag for the purification of recombinant protein via Ni-NTA resins. The 6xHistidine-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

Important licensing information

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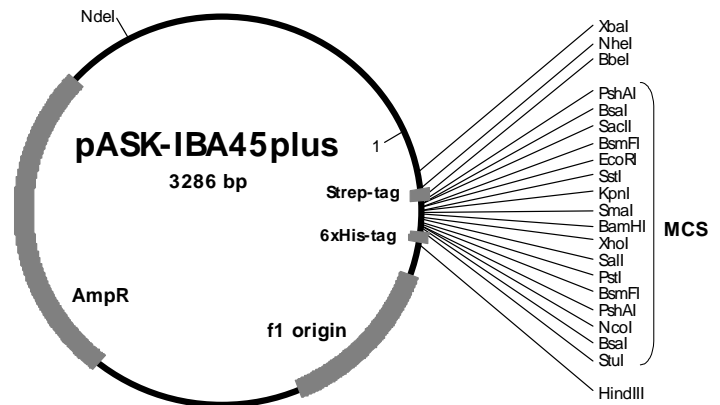
Multiple Cloning Site of pASK-IBA45plus

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC	79
	forward primer	
80	AGTGATAGAGAAAAGTGAATAGTAGTTCGACAAAAATCTAGAATAATTTTTGTTTAACTTTAAGAAGGAGATATACAA	159
	XbaI	
	link Strep-tag link	
	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	ATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgCGAGACCGCGTCCCGAATTCGAGCTCGGTACCCGGGGATCC	239
	NheI BbeI BsaI BsmFI SstI KpnI BamHI	
	EheI PshAI EcoRI SmaI	
	KasI SacII	
	NarI	
	6xHistidine-tag	
	S R S T C R G T M V S G L R G S H H H H H H *	
240	CTCGAGGTCGACCTGCAGGGGACCATGGTCTCaggccTGAGAGGATCGCATCACCATCACCATCAATAAGCTTGAC	319
	XhoI SalI PstI BsmFI BsaI StuI HindIII	
	PshAI	
	NcoI	
320	CTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTGCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACG	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-IBA45plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
multiple cloning site	193	279
6xHistidine-tag	280	309
reverse primer binding site	368	384
f1 origin	397	835
AmpR resistance gene	984	1844
Tet-repressor	1854	2477
Col E1 origin	2630	3218



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