

Data Sheet

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pASK-IBA33plus

Cat. No. : 2-1433-000

Lot No.: 1433-

Last date of revision
May 10

Version 1433-9

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	6xHistidine-tag 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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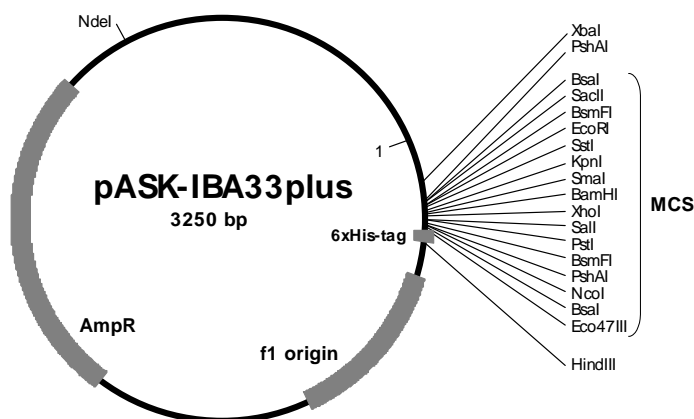
Multiple Cloning Site of pASK-IBA33plus

1	CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATGATAGAGTTATTTTACCACTCCCTA	77
	forward primer	
78	TCAGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATAC	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	AaatgGGAGACCGCGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTC	237
	BsaI BsmFI SstI KpnI BamHI SalI PstI BsmFI BsaI PshAI EcoRI SmaI XhoI PshAI SacII NcoI	
	6xHistidine-tag S A R G S H H H H H H *	
238	AgcgcTAGAGGATCGCATCACCATCACCATCACTAATAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACA	317
	Eco47III HindIII	
318	TTTTTTTGTCTGCCGTTTACCGCTACTGCGTACACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGGTGT	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-IBA33plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
multiple cloning site	160	243
6xHistidine-tag	244	273
reverse primer binding site	332	348
f1 origin	361	799
AmpR resistance gene	948	1808
Tet-repressor	1818	2441
Col E1 origin	2594	3182



<p>Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i></p> <p>Forward: 5' - NNNNNNGGTCTCNA ATG ^(N₁₇)* ...</p> <p>Reverse: 5' - NNNNNNGGTCTCNGC GCT ^(N₂₀) ...</p>	<p>Sequencing primers:</p> <p>Forward: 5' - GAGTTATTTTACCACTCCCT -3'</p> <p>Reverse: 5' - CGCAGTAGCGGTAAACG -3'</p>
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* The ATG start codon is already included