

Data Sheet

pEXPR-IBA44

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Description	Eukaryotic expression vector designed for high-level stable and non-replicative transient expression in most mammalian hosts containing the following elements: <ul style="list-style-type: none"> • Human cytomegalovirus (CMV) immediate-early promoter for high-level expression in a wide range of mammalian cells • Multiple cloning site • Neomycin resistance gene for selection of stable cell lines • Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7) • The expressed recombinant protein will be secreted into the medium. • <i>Strep</i>-Tactin affinity tag (<i>Strep</i>-tag II) for the purification of recombinant protein via <i>Strep</i>-Tactin resins. The <i>Strep</i>-tag is fused to the N-terminus of the recombinant protein. • 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.
Resistance for selection in E. coli	Ampicillin
Form	5 µg, dissolved in TE buffer (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
Shipment	Room temperature

For research use only

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. *Strep*-tag® and *Strep*-Tactin® are registered trademarks of IBA GmbH. The 6xHistidine-tag is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche Ltd., Basel, Switzerland and is provided only for the use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

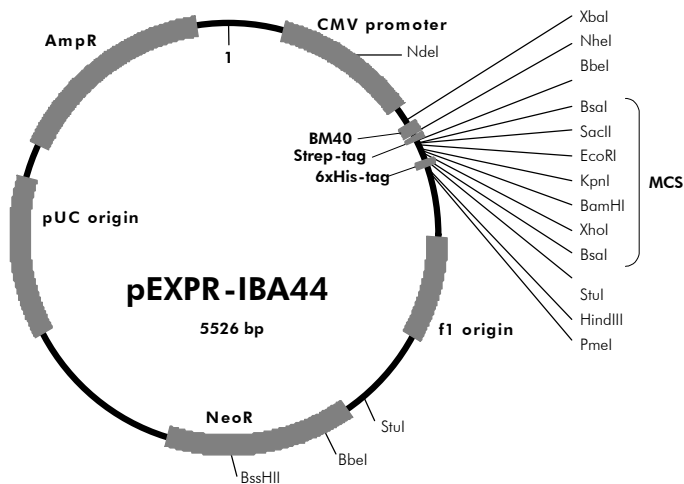
Multiple Cloning Site of pEXPR-IBA44

721	AAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTCACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAG	800
	CAAT	
801	GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG	880
	TATA forward primer	
	BM40 link	
	M R A W I F F L L C L A G R A L A A S W S H	
881	GGTCTAGACCCACCATGAGGGCCTGGATCTTCTTTCTCCTTTGCTGGCCGGGAGGGCTCTGGCAGCTAGCTGGAGCCAC	960
	XbaI NheI	
	Strep-tag link	
	P Q F E K G A E T A V P N S S S V P G D P S R S T C R	
961	CCGCAGTTCGAAAAAGgcgCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGATCCCTCGAGGTCGACCTGCAG	1040
	BbeI BsaI EcoRI KpnI BamHI XhoI	
	EheI SacII	
	KasI	
	NarI	
	6xHistidine-tag	
	G T M V S G L R G S H H H H H *	
1041	GGGACCATGGTCTCaggccTGAGAGGATCGCATCACCATCCTAATAAGCTTAAGTTAAACCGCTGATCAGC	1120
	BsaI StuI HindIII PmeI	
1121	CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCTCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTC	1200
	reverse primer	

Please note: Restriction enzymes in bold cut twice, however, not all multiple cutting restriction enzymes are shown. 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 pASK-IBA vectors for bacterial expression. During secretion of the recombinant protein into the medium, the BM40 signal sequence will be cleaved off. The processed protein will start with the Ala - Ser – linker.

Features of pEXPR-IBA44

	from bp	to bp
CMV promoter	232	819
forward primer binding site	832	852
BM40 signal sequence	895	945
Strep-tag	955	975
multiple cloning site	976	1062
6xHistidine-tag	1063	1092
reverse primer binding site	1120	1137
f1 origin	1396	1824
Neomycin resistance gene	2234	3028
pUC origin	3715	4385
Ampicillin resistance gene	4530	5390



Cloning primers for the precise cloning using *BsaI* or *Eco31I*

Forward: 5'-NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN ...

Reverse: 5'-NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN ...

Sequencing primers:

Forward: 5'- GAGAACCCTGCTTACTGGC -3'

Reverse: 5'- TAGAAGGCACAGTCGAGG -3'