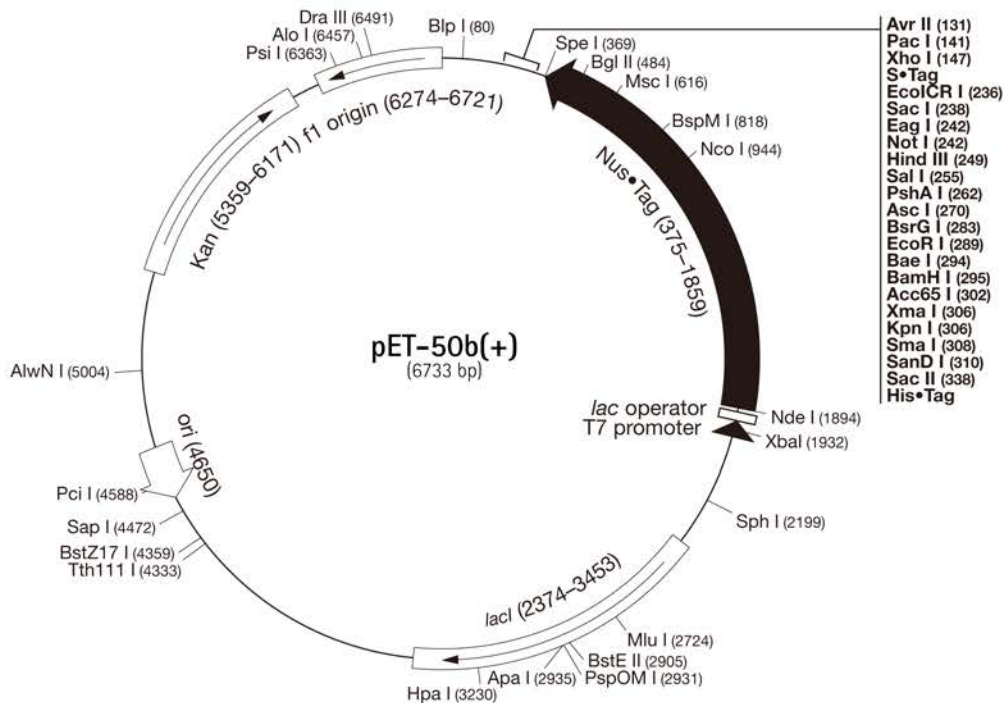


pET-50b(+) Vector

	Cat. No.
pET-50b(+) DNA	71464-3
pET-50b(+) sequence landmarks	
T7 promoter	1967-1983
T7 transcription start	1966
His•Tag coding sequence	1866-1883
Nus•Tag coding sequence	375-1859
His•Tag coding sequence	342-359
Multiple cloning sites (<i>SanD</i> I - <i>Avr</i> II)	131-315
S•Tag coding sequence	168-212
T7 terminator	26-73
<i>lacI</i> coding sequence	2374-3453
pBR322 ori	4650
Kan coding sequence	5359-6171
f1 origin	6274-6721

The pET-50b(+) vector carries N-terminal Nus•Tag™ and His•Tag® coding sequences followed by a recognition site for the human rhinovirus (HRV) 3C protease. This protease is highly specific for cleavage of the sequence LEVLFQ↓GP (1), and is active at low temperatures (2). pET-50b(+) also contains an optional C-terminal thrombin recognition site followed by an S•Tag™ coding sequence. Unique restriction sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with the helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the AS S•Tag 18mer Primer (Cat. No. 71262-3).

1. Cordingley, M.G., Register, R.B., Callahan, P.L., Garsky, V.M., and Colonno, R.J. (1989) *J. Virol.* 63, 5037-5045.
2. Wang, Q.M., Johnson, R.B., Cox, G.A., Villarreal, E.C., and Loncharich, R.J. (1997) *Anal. Biochem.* 252, 238-245.



T7 Promoter Primer
#69348-3

T7 promoter → lac operator Xba I

GATGCGTCCGGCGTAGAGGATCGAGATCGATCTCGATCCCGCAAATAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCTCTAGA

His•Tag Nus•Tag

AATAATTTTGTAACTTTAAGAAGGAGATATACATATGGGCAGCAGCCATCACCACCATCACCATTCTAGTATG...1443bp...ATGGCTGCCGT
MetGlySerSerHisHisHisHisHisHisSerSerMet...481 aa...MetAlaAlaArg

Acc65 I
Kpn I
Bae I
SanD I
Xma I
Sma I

Nus•Tag His•Tag Sac II

AATATTTGCTGGTTCGGTGACGAAGCGACTAGTGGTTCGGTCATCACCATCACCATCACTCCGCGGCTCTTGAAGTCTCTTTACAGGGACCCGGGTAC
AsnIleCysTrpPheGlyAspGluAlaThrSerGlySerGlyHisHisHisHisHisHisSerAlaAlaLeuGluValLeuPheGlnGlyProGlyTyr

HRV 3C I

S•Tag

BamH I EcoR I BsrG I Asc I PshA I Sal I Hind III Eag I EcoICR I Sac I

CAGGATCCGAATTCTGTACAGGCCCTTGGCGCGCCGACGTCGCTGACAAAGCTTGGCGCGCAGAGCTCGCTCTGGTGCCACGCGGTAGTAAAGAAACC
GlnAspProAsnSerValGlnAlaLeuAlaArgProThrSerValAspLysLeuAlaAlaAlaGluLeuAlaLeuValProArgGlySerLysGluThr

thrombin I

S•Tag Xho I Pac I Avr II

GCTGCTGCTAAATTCGAACGCCAGCATGGACAGCTCTACTTCTGCTGCTCTCGAGGCTTAATTAACCTAGGCTGCTAAACAAAGCCCGAAAGGAAGC
AlaAlaAlaLysPheGluArgGlnHisMetAspSerSerThrSerAlaAlaLeuGluAlaEnd

AS S•Tag 18mer Primer
#71262-3

pET-50b(+) cloning/expression region

pET-50b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AatII	2	266 1729	BstXI	3	2526 2655 2778	Tth1111	1	4333			
Acc65I	1	302	BstYI	9	295 484 1399 2288 3500	XbaI	1	1932			
AccI	3	256 1070 4358	BstZ17I	1	3780 5229 5240 6039	XcmI	3	2580 3096 3114			
AcII	3	2386 3913 6276	BtgI	4	335 505 944 2161	XhoI	1	147			
AfeI	2	2129 3842	BtsI	5	648 3089 3457 5651 5738	XmaI	1	306			
AfIII	2	2724 4588	Clal	5	1045 1123 1711 2001 5481	XmnI	3	1792 4146 6179			
AleI	2	1441 1458	DrallI	1	6491	ZraI	2	264 1727			
Alol	1	6457	DrdI	3	4281 4696 6446	Enzymes that do not cut pET-50b(+):					
AlwNI	1	5004	EaeI	7	242 512 614 901 2032	AarI	AfIII	AgeI	AhdI	AsiSI	BbvCI
Apal	1	2935	EagI	1	2164 3398	BglI	BmgBI	BmtI	BpII	BsaI	BseRI
ApaI	3	2704 4402 4902	EarI	3	242	BsiWI	Bsu36I	BtrI	DraI	FalI	FseI
AscI	1	270	EarI	3	2342 4472 5603	FspAI	Fspl	MfeI	NheI	PinAI	PmeI
Asel	5	1981 3409 3468 5989 6178	Ecil	5	798 1353 2515 4650 4796	PmlI	Psrl	PstI	RsrII	SbfI	Scal
Aval	2	147 306	Eco57I	2	1367 5136	SexAI	Sfil	SnaBI	SrfI	Sse8387I	Swal
AvrII	1	131	Eco57MI	5	1367 2562 3051 4115 5136						
BaeI	1	294	EcoCRI	1	236						
BamHI	1	295	EcoNI	3	318 2259 5702						
BanI	10	223 302 991 2046 2067	EcoO109I	4	53 310 2157 3594						
BanII	6	2181 2644 3363 3493 6528	EcoRI	1	289						
		238 2108 2122 2935 5445	EcoRV	2	452 1043						
		6566	HaeII	14							
BbeI	4	2050 2071 2185 3367	HincII	4	257 441 836 3230						
BbsI	4	749 2870 3209 3706	HindIII	1	249						
BceAI	11		HpaI	1	3230						
BcgI	3	236 3050 4165	KasI	4	2046 2067 2181 3363						
BciVI	4	1815 3181 4791 6185	KpnI	1	306						
BclI	2	1474 2738	MluI	1	2724						
BfrBI	2	5638 5904	MscI	1	616						
BglII	1	484	MslI	8	1441 1458 2776 3064 3094						
BlpI	2	80 819			3575 3770 4161						
BmeI580I	4	2708 2935 4406 4906	NaeI	2	2036 6594						
BmriI	6	1055 2253 2650 2887 3527	NarI	4	2047 2068 2182 3364						
		4327	NcoI	1	944						
Bpml	3	2562 3051 4115	NdeI	1	1894						
Bpu10I	2	3694 5807	NgoMIV	2	2034 6592						
BpuEI	5	21 3538 4679 4977 5218	NotI	1	242						
BsaAI	2	4340 6491	NruI	4	751 1176 1800 5447						
BsaBI	3	1997 2007 3785	Nsil	2	5640 5906						
BsaHI	7	263 1726 2047 2068 2182	NspI	4	2199 3933 4225 4592						
		2681 3364	NspV	2	187 1786						
BsaWI	8	2 1117 3043 3546 3777	Pacl	1	141						
		4794 4941 5925	Pcil	1	4588						
BsaXI	3	331 3399 6455	PfiMI	3	179 2306 6053						
BseYI	4	1649 3123 3258 4892	PfoI	2	2291 4230						
BsgI	4	1497 2575 2775 3748	Ppil	2	5303 6457						
BsiEI	8	245 656 794 1330 1711	PpuMI	2	310 3594						
		3509 4504 4928	PshAI	1	262						
BsiHKAI	8	238 423 1315 2224 2708	PsiI	1	6363						
		3582 4406 4906	PspOMI	1	2931						
BsmAI	6	2421 2826 2952 3339 4229	PvuI	2	794 1711						
		5806	PvuII	3	3324 3417 4179						
BsmBI	3	3339 4229 5806	SacI	1	238						
BsmFI	4	296 2185 3859 6706	SacII	1	338						
BsmI	3	528 5674 5751	Sall	1	255						
Bsp1286I	13		SanDI	1	310						
BspCNI	10	93 114 878 3309 3686	SapI	1	4472						
		3848 4388 4876 5285 5820	Sfcl	5	1835 1966 4853 5044 6710						
BspEI	2	2 3777	Sfol	4	2048 2069 2183 3365						
BspHI	3	2122 5308 6183	SgrAI	2	1440 2043						
BspLU111	1	4588	SmaI	1	308						
BspMI	1	818	SmlI	6	36 147 3517 4694 4956						
BsrBI	5	864 1953 4521 6189 6635			5233						
BsrDI	4	614 995 2771 3137	SpeI	1	369						
BsrFI	9	568 924 988 1440 2034	SphI	1	2199						
		2043 2410 5744 6592	SspI	3	398 5715 6283						
BsrGI	1	283	StuI	2	280 669						
BssHII	5	270 1088 1280 1769 3135	StyI	5	57 131 275 944 1640						
BssSI	2	1160 4761	TaqII	5	426 3523 4490 6044 6395						
Bst1107I	1	4359	TatI	2	283 4392						
BstAPI	3	985 1511 2407	TspGWI	9	274 1245 1318 1482 1695						
BstBI	2	187 1786			3713 4031 5599 5611						
BstEII	1	2905									