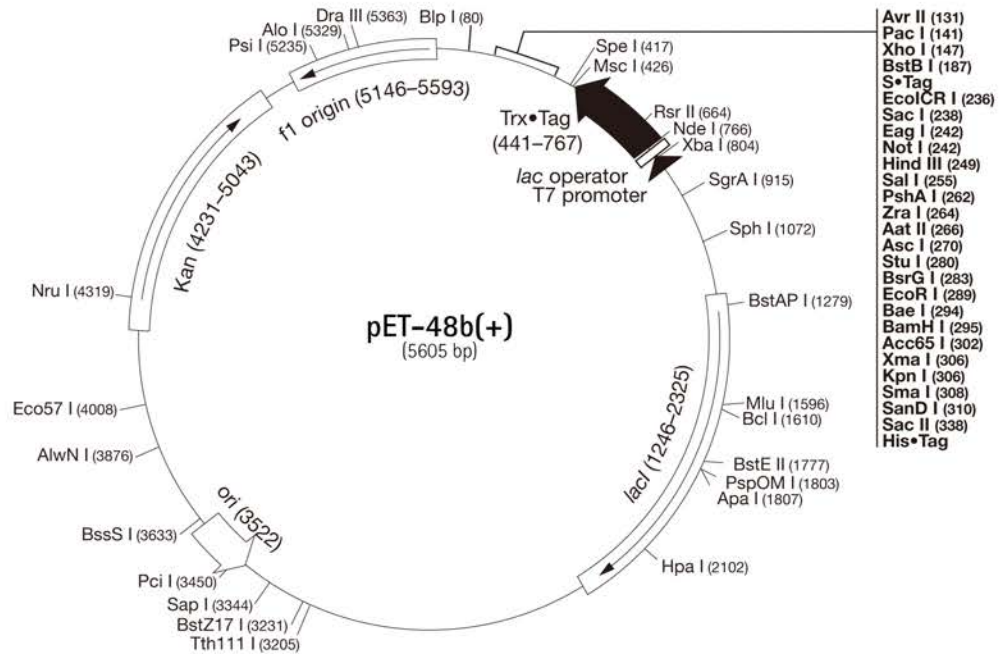


pET-48b(+)⁺ Vector

	Cat. No.
pET-48b(+) ⁺ Cloning Kit	71462-3
pET-48b(+)⁺ sequence landmarks	
T7 promoter	839-855
T7 transcription start	838
Trx•Tag coding sequence	441-767
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	1246-2325
pBR322 ori	3522
Kan coding sequence	4231-5043
f1 origin	5146-5593

The pET-48b(+)⁺ vector carries N-terminal Trx•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 LEVL^{FQ}↓GP (1), and is active at low temperatures (2). pET-48b(+)⁺ 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.



#69348-3
 TCCGGCGTAGAGGATCGAGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAAT

T7 promoter → lac operator Xba I

TTTGTTTAACTTTAAGAAGGAGATATACATATGAGCGAT...309 bp...AACCTGGCCGGTTCTGGTCTGGCCATACTAGTGGTGGTGGCGGTTCT
 MetSerAsp...103 aa...AsnLeuAlaGlySerGlySerGlyHisThrSerGlyGlyGlyGlySer

Nde I Trx•Tag Msc I Spe I

AATAACAATCCTCTACTCTACTCCATCTAGTGGTTCTGGTCATCACCATCACCATCACTCCGCGGCTCTTGAAGTCTCTTTCAAGGACCCGGGTAC
 AsnAsnAsnProProThrProThrProSerSerGlySerGlyHisHisHisHisHisSerAlaAlaLeuGluValLeuPheGlnGlyProGlyTyr

His•Tag Sac II HRV 3C

BamH I EcoR I BsrG I Stu I Asc I PshA I Eag I EcoI CR I S•Tag
 CAGGATCCGAATTCGTACAGGCCCTTGGCGCGCCGACGTCGCTCGACAAGCTTGGCGCCGACAGCTCGCTCTGGTGCACGCGGTAGTAAAGAAACC
 GlnAspProAsnSerValGlnAlaLeuAlaArgProThrSerValAspLysLeuAlaAlaAlaGluLeuAlaLeuValProArgGlySerLysGluThr

S•Tag BstB I Xho I Pac I Avr II thrombin I

GCTGCTGCTAAATTCGAACGCCAGCACATGGACGCTCTACTTCTGTCTCGAGGCTTAATTAACCTAGGCTGCTAAACAAAGCCGAAAGGAAGC
 AlaAlaAlaLysPheGluArgGlnHisMetAspSerSerThrSerAlaAlaLeuGluAlaEnd

AS S•Tag 18mer Primer #71262-3

pET-48b(+)⁺ cloning/expression region

pET-48b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AatII	1	266	BtgI	3	335 544 1033	ZraI	1	264			
Acc65I	1	302	BtsI	5	572 1961 2329 4523 4610	Enzymes that do not cut pET-48b(+):					
AccI	2	256 3230	Clal	3	583 873 4353	AarI	AfIII	AgeI	AhdI	AleI	AsiSI
AccII	3	1258 2785 5148	DrallI	1	5363	BbvCI	BglI	BglII	BmgBI	BmtI	BpII
AfeI	2	1001 2714	DrdI	3	3153 3568 5318	Bsal	BseRI	BsiWI	BspMI	Bsu36I	BtrI
AfIII	2	1596 3460	EaeI	6	242 424 440 904 1036	DraI	EcoRV	FalI	FseI	FspAI	FspI
Alol	1	5329			2270	MfeI	NcoI	NheI	PinAI	PmeI	PmlI
AlwNI	1	3876	EagI	1	242	Psrl	PstI	PvuI	SbfI	Scal	SexAI
Apal	1	1807	EarI	3	1214 3344 4475	Sfil	SnaBI	SrfI	Sse8387I	Swal	
ApaLI	4	485 1576 3274 3774	Ecil	3	1387 3522 3668						
AscI	1	270	Eco57I	1	4008						
AseI	5	853 2281 2340 4861 5050	Eco57MI	4	1434 1923 2987 4008						
AvaI	2	147 306	EcoCRI	1	236						
AvrII	1	131	EcoNI	3	318 1131 4574						
BaeI	1	294	EcoO109I	4	53 310 1029 2466						
BamHI	1	295	EcoRI	1	289						
BanI	9	223 302 918 939 1053	HaeII	13							
		1516 2235 2365 5400	HincII	2	257 2102						
BanII	6	238 980 994 1807 4317	HindIII	1	249						
		5438	HpaI	1	2102						
BbeI	4	922 943 1057 2239	KasI	4	918 939 1053 2235						
BbsI	3	1742 2081 2578	KpnI	1	306						
BceAI	6	1115 1455 2082 3962 4981	MluI	1	1596						
		5388	MscI	1	426						
BcgI	5	236 516 673 1922 3037	MslI	7	420 1648 1936 1966 2447						
BciVI	4	533 2053 3663 5057			2642 3033						
BclI	1	1610	NaeI	2	908 5466						
BfrBI	2	4510 4776	NarI	4	919 940 1054 2236						
BlpI	1	80	NdeI	1	766						
Bme1580I	5	489 1580 1807 3278 3778	NgoMIV	2	906 5464						
Bmrl	5	1125 1522 1759 2399 3199	NotI	1	242						
Bpml	3	1434 1923 2987	NruI	1	4319						
Bpu10I	2	2566 4679	Nsil	2	4512 4778						
BpuEI	5	21 2410 3551 3849 4090	Nspl	4	1071 2805 3097 3464						
BsaAI	2	3212 5363	NspV	1	187						
BsaBI	3	869 879 2657	Pacl	1	141						
BsaHI	6	263 919 940 1054 1553	Pcil	1	3460						
		2236	PfiMI	4	179 373 1178 4925						
BsaWI	7	2 1915 2418 2649 3666	PfoI	2	1163 3102						
		3813 4797	Ppil	2	4175 5329						
BsaXI	4	331 368 2271 5327	PpuMI	2	310 2466						
BseYI	3	1995 2130 3764	PshAI	1	262						
BsgI	3	1447 1647 2620	Psil	1	5235						
BsiEI	4	245 2381 3376 3800	PspOMI	1	1803						
BsiHKAI	7	238 489 1096 1580 2454	PvuII	3	2196 2289 3051						
		3278 3778	RsrII	1	664						
BsmAI	6	1293 1698 1824 2211 3101	SacI	1	238						
		4678	SacII	1	338						
BsmBI	3	2211 3101 4678	Sall	1	255						
BsmFI	4	296 1057 2731 5578	SanDI	1	310						
BsmI	2	4546 4623	SapI	1	3344						
Bsp1286I	12		Sfcl	4	838 3725 3916 5582						
BspCNI	9	93 114 2181 2558 2720	SfoI	4	920 941 1055 2237						
		3260 3748 4157 4692	SgrAI	1	915						
BspEI	2	2 2649	SmaI	1	308						
BspHI	3	994 4180 5055	SmlI	6	36 147 2389 3566 3828						
BspLU11I	1	3460			4105						
BsrBI	4	825 3393 5061 5507	SpeI	1	417						
BsrDI	2	1643 2009	SphI	1	1071						
BsrFI	6	438 906 915 1282 4616	SspI	2	4587 5155						
		5464	StuI	1	280						
BsrGI	1	283	StyI	3	57 131 275						
BssHII	2	270 2007	TaqII	4	2395 3362 4916 5267						
BssSI	1	3633	TatI	3	283 715 3264						
Bst1107I	1	3231	TspGWI	8	274 561 677 711 2585						
BstAPI	1	1279			2903 4471 4483						
BstBI	1	187	Tth111I	1	3205						
BstEII	1	1777	XbaI	1	804						
BstXI	4	422 1398 1527 1650	XcmI	4	419 1452 1968 1986						
BstYI	7	295 1160 2372 2652 4101	XhoI	1	147						
		4112 4911	XmaI	1	306						
BstZ17I	1	3231	XmnI	3	463 3018 5051						