

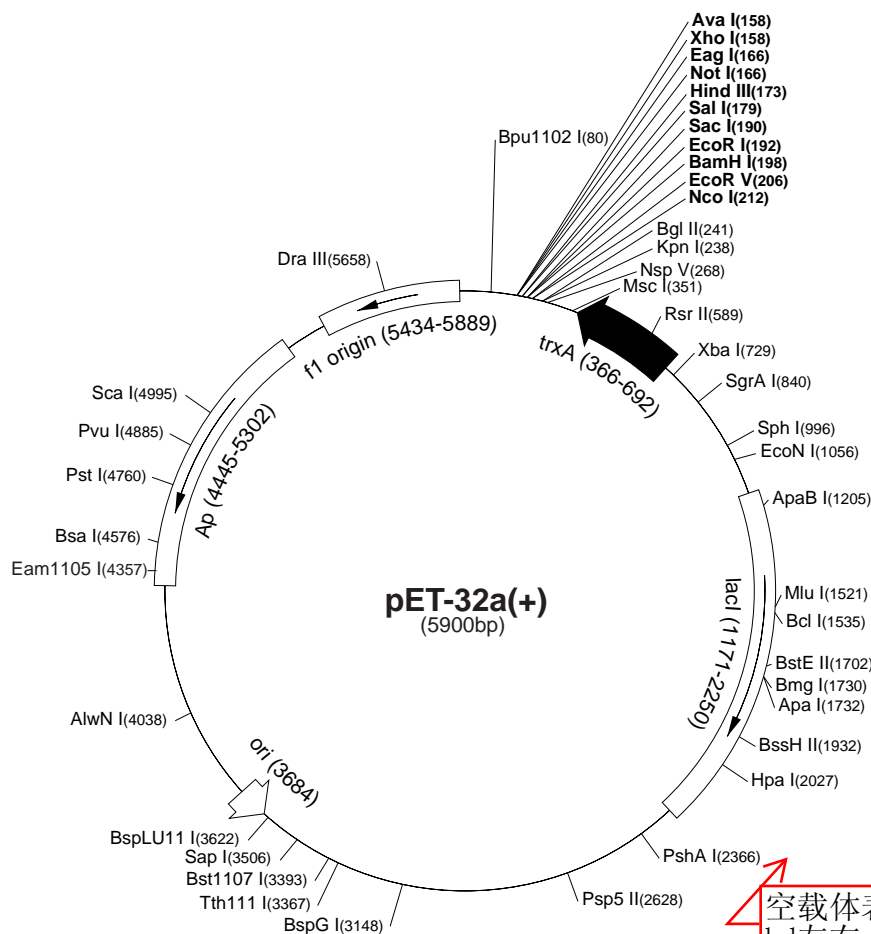
	Cat. No.
pET-32a DNA	69015-3
pET-32b DNA	69016-3
pET-32c DNA	69017-3

The pET-32 series is designed for cloning and high-level expression of peptide sequences fused with the 109aa Trx•Tag™ thioredoxin protein (1). Cloning sites are available for producing fusion proteins also containing cleavable His•Tag® and S•Tag™ sequences for detection and purification. Unique 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 helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

1. LaVallie, E.R., DiBlasio, E.A., Kovacic, S., Grant, K.L., Schendel, P.F. and McCoy, J.M. (1993) *BioTechnology* **11**, 187-193.

pET-32a(+) sequence landmarks	
T7 promoter	764-780
T7 transcription start	763
Trx•Tag coding sequence	366-692
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites ( <i>Nco</i> I - <i>Xho</i> I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	1171-2250
pBR322 origin	3684
<i>bla</i> coding sequence	4445-5302
f1 origin	5434-5889

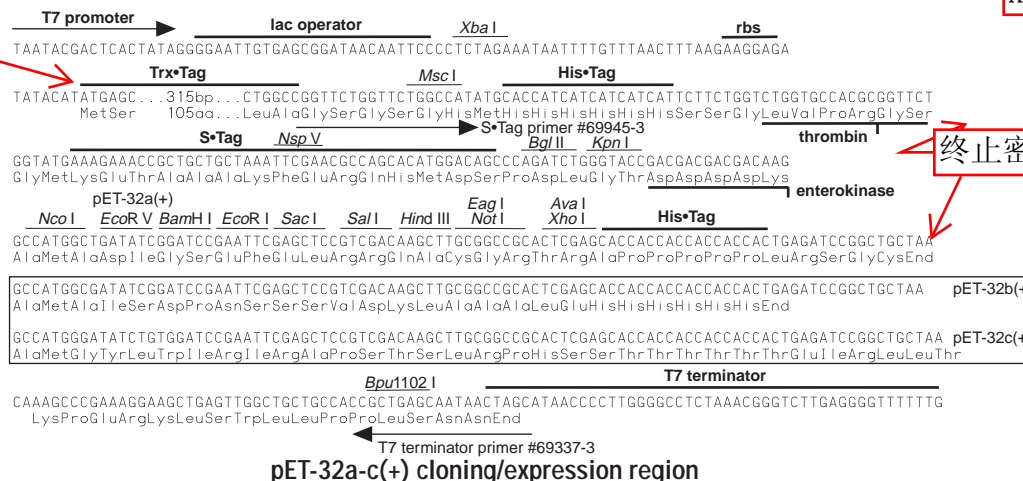
The maps for pET-32b(+) and pET-32c(+) are the same as pET-32a(+) (shown) with the following exceptions: pET-32b(+) is a 5899bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-32c(+) is a 5901bp plasmid; add 1bp to each site beyond *Bam*H I at 198 except for *Eco*R V, which cuts at 209.



空载体表达蛋白24  
kd左右

## 起始密码子

终止密码子



# pET-32a(+) Restriction Sites

TB122 12/98

Enzyme	# Sites	Locations					
AccI	2	180	3392				
AccIII	8	1288	2016	2347	3131	3272	
		3574	4814	5498			
Acil	85						
AflIII	2	1521	3622				
AluI	25						
AlwI	17						
Alw21I	10	159	190	414	1021	1505	
		2616	3440	3940	5101	5186	
Alw44I	5	410	1501	3436	3936	5182	
AlwNI	1	4038					
Apal	1	1732					
ApaBI	1	1205					
ApoI	5	192	270	1796	5460	5471	
AvaI	1	158					
Avall	8	589	2073	2449	2537	2628	
		2907	4653	4875			
BamHI	1	198					
BanI	11						
BanII	5	190	905	919	1732	5733	
BbsI	4	1667	2006	2380	2740		
BbvI	29						
BccI	14						
Bce83I	7	21	2335	2505	3713	4011	
		4252	5120				
Bcefl	5	1040	1381	2008	4124	5684	
BcgI	14						
BclI	1	1535					
Bfal	7	70	730	2636	4117	4370	
		4705	5809				
BglI	2	2585	4635				
BglII	1	241					
BmgI	1	1730					
BpmI	5	1359	1848	2482	3149	4585	
Bpu10I	1	2728					
Bpu1102I	1	80					
BsaI	1	4576					
BsaAI	2	3374	5658				
BsaBI	3	794	804	2819			
BsaHI	6	844	865	979	1478	2161	
		5052					
BsaJI	9	57	212	469	496	958	
		964	2156	2594	3782		
BsaWI	7	2	1840	2343	2811	3828	
		3975	4806				
BsaXI	2	2180	5606				
Bsbl	2	3338	5565				
BscGI	14						
BsgI	3	1372	1572	2782			
Bsil	2	3795	5179				
BsiEI	6	169	2306	3538	3962	4885	
		5034					
BsII	25						
BsmAI	7	1218	1623	1749	2136	3263	
		4576	5352				
BsmBI	2	2136	3263				
BsmFI	4	982	2523	2893	5873		
BsoFI	50						
Bsp24I	10	811	843	1362	1394	1664	
		1696	4115	4147	4293	4325	
Bsp1286I	14						
BspEI	2	2	2811				
BspGI	1	3148					
BspLU11I	1	3622					
BsrI	25						
BsrBI	4	750	3555	5356	5802		
BsrDI	4	1568	1934	4576	4750		
BsrFI	8	363	831	840	1207	2419	
		2579	4595	5759			
BssHII	1	1932					
Bst1107I	1	3393					
BstEII	1	1702					

Enzyme	# Sites	Locations					
BstXI	3	1323	1452	1575			
BstYI	12						
CacBI	39						
CjeI	26						
CjePI	18						
Clal	2	508	798				
CviJI	90						
CvIRI	28						
DdeI	11						
DpnI	32						
DraI	3	4381	4400	5092			
DraIII	1	5658					
DrdI	3	3315	3730	5613			
DrdII	3	357	1244	5663			
Dsal	4	212	469	958	2594		
EaeI	7	166	349	365	829	961	
		2195	4903				
EagI	1	166					
Eam1105I	1	4515					
EarI	3	1139	3506	5310			
EcII	4	1298	3696	3842	4670		
Eco47III	3	926	2427	2876			
Eco57I	2	4170	5182				
EcoNI	1	1056					
EcoO109I	3	53	954	2628			
EcoRI	1	192					
EcoRII	9	367	495	1244	1559	2099	
		2156	3648	3769	3782		
EcoRV	1	206					
FauI	17						
FokI	13						
FspI	2	2603	4737				
GdIII	6	166	365	829	961	2195	
		4903					
HaeI	7	217	351	1249	2570	3637	
		3648	4100				
HaeII	14						
HaeIII	27						
HgaI	13						
HgiEII	3	419	1119	4208			
HhaI	46						
HinAI	4	203	1420	4514	4588		
HincII	2	181	2027				
HindIII	1	173					
HinfI	16						
HpaI	1	2027					
HphI	18						
KpnI	1	238					
MaeI	15						
MaeII	18						
MbolI	15						
MluI	1	1521					
MmeI	3	3837	4021	5635			
MnlI	28						
MscI	1	351					
MseI	28						
MslI	9	1573	1861	1891	2609	2804	
		3195	4767	4926	5285		
MspI	32						
MspAII	10	84	283	1551	2121	2214	
		3213	3332	3964	4209	5150	
MwoI	41						
NarI	4	844	865	979	2161		
NciI	12						
NcoI	1	212					
NdeI	2	346	691				
NgoAIV	4	831	2419	2579	5759		
NlaIII	26						
NlaIV	27						
NotI	1	166					
NspI	4	996	2967	3259	3626		
NspV	1	268					

Enzyme	# Sites	Locations					
Pfi1108I	2	2408	4533				
PfiMI	2	260	1103				
PleI	10	466	778	1070	1157	1953	
		3516	4001	4504	5593	5601	
PshAI	1	2366					
Psp5II	1	2628					
Psp1406I	6	1183	2551	2947	4741	5114	
		5443					
PstI	1	4760					
PvuI	1	4885					
PvuII	3	2121	2214	3213			
RcaI	3	919	4342	5350			
RsaI	5	236	642	1668	3428	4995	
RsrII	1	589					
SacI	1	190					
Sall	1	179					
SapI	1	3506					
Sau96I	19						
Sau3AI	32						
Scal	1	4995					
ScrFI	21						
SfaNI	21						
Sfcl	5	763	3887	4078	4756	5877	
SgrAI	1	840					
SphI	1	996					
Sspl	2	5319	5450				
StyI	2	57	212				
TaqI	18						
TaqII	10	429	1429	1647	2320	3524	
		4863	5048	5201	5218	5562	
TfiI	6	566	2200	2502	2672	3176	
		3597					
Thal	35						
TseI	29						
Tsp45I	8	1702	2530	3061	3274	3369	
		4771	4982	5831			
Tsp509I	18						
Tth111I	1	3367					
Tth111II	6	1360	2053	3083	4212	4219	
		4251					
UbaII	21						
VspI	4	778	2206	2265	4687		
XbaI	1	729					
XcmI	3	1377	1893	1911			
XhoI	1	158					
XmnI	3	388	3180	5114			

Enzymes that do not cut pET-32a(+):

AatII	AflII	AgeI	AscI	AvrII
BaeI	BseRI	BsmI	BspMI	BsrGI
Bsu36I	FseI	MunI	NheI	NruI
NsiI	PacI	PmeI	PmlI	RleAI
SacII	SexAI	SfiI	SgfI	SmaI
SnaBI	SpeI	SrfI	Sse8387I	StuI
SunI	Swal			