

pET Directional TOPO® Expression Kits

Simplified Directional Cloning and High-level Expression in *E. coli*

Description:

The pET Directional TOPO® Expression Kits feature linearized, topoisomerase I-activated pET expression vectors for five-minute directional cloning and subsequent high-level expression in *E. coli*. Directional TOPO® Cloning technology facilitates gene expression experiments because:

- A proofreading enzyme is used for PCR, resulting in fewer errors in cloned genes
- Greater than 90% of the clones are in the correct orientation for gene expression, reducing the time spent on colony screening

Five pET Directional TOPO® Expression Vectors are available (Figure 1 and Table 1): pET100/D-TOPO®, pET101/D-TOPO®, pET102/D-TOPO®, pET151/D-TOPO®, and pET200/D-TOPO®. Each vector carries a T7/*lac* promoter for high-level expression. The T7/*lac* promoter contains a *lac* operator sequence immediately downstream of the strong T7 promoter for added regulation of basal expression. All vectors carry the low-copy pBR322 origin of replication to further reduce basal expression levels in the uninduced state. In addition, each vector offers flexible options for simplifying protein detection, cleaving purification tags, selecting plasmid-carrying clones, and/or improving protein yields.

Figure 1 - pET Directional TOPO® vectors

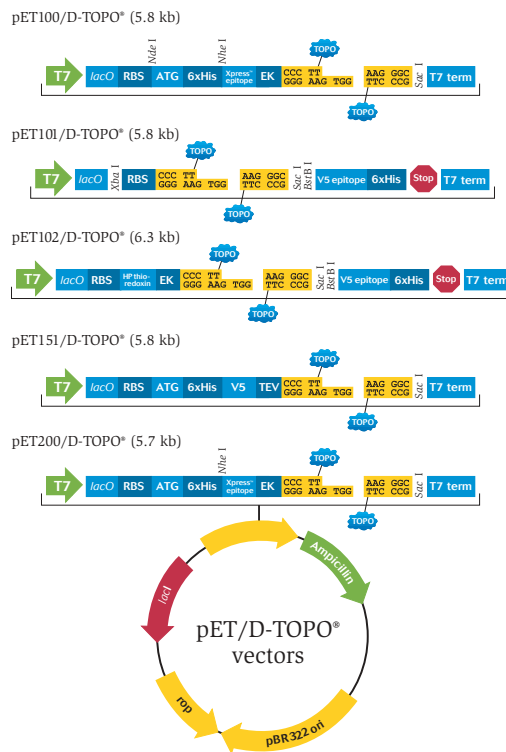


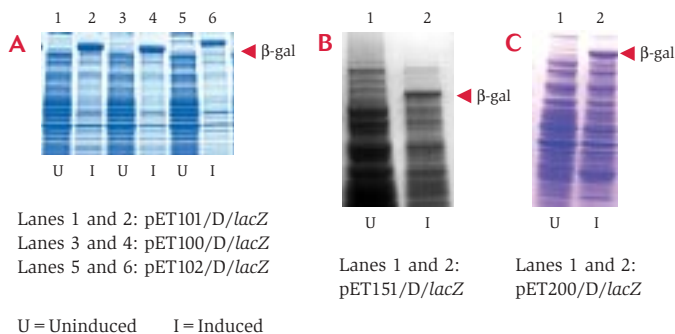
Table 1 - Features of the pET Directional TOPO® expression vectors

Vector	Fusion partner		Cleavage protease	Antibiotic resistance	Advantage
	Position	Tag			
pET100/D-TOPO®	N-term	Xpress™, 6xHis	EK	Amp	Cleavable detection and purification tag
pET101/D-TOPO®	C-term	V5, 6xHis	N/A	Amp	Detection and purification tag
pET102/D-TOPO®	N-term	thioredoxin	EK	Amp	Cleavable thioredoxin tag enhances protein translation and solubility
	C-term	V5, 6xHis	N/A	Amp	Detection and purification tag
pET151/D-TOPO®	N-term	V5, 6xHis	TEV	Amp	Cleavable detection and purification tag
pET200/D-TOPO®	N-term	Xpress™, 6xHis	EK	Kan	Cleavable detection and purification tag

pET Directional TOPO® Expression Kits, continued

With the Directional TOPO®-adapted pET vectors you can expect the same proven expression achieved with the original pET vectors. Figure 2 shows expression of the *lacZ* gene in all five pET Directional TOPO® vectors. Figure 3 demonstrates efficient cleavage of the N-terminal tag using TEV in pET151/D-TOPO®.

Figure 2 - Expression in Directional TOPO®-adapted pET vectors



The *lacZ* gene was directionally TOPO® Cloned into pET100/D-TOPO®, pET101/D-TOPO®, pET102/D-TOPO®, pET151/D-TOPO®, and pET200/D-TOPO®. Constructs were transformed into BL21 Star™(DE3) *E. coli*. A single colony from each transformation was picked and grown in LB medium supplemented with the appropriate antibiotic to OD₆₀₀ = 0.5. Two and one-half hours post-induction with 1 mM IPTG, cultures were harvested and a portion analyzed on a 4-20% Novex® Tris-Glycine gel.

Figure 3 - Cleavage of the N-terminal V5-6xHis tag from β-gal expressed in the pET151/D-TOPO® vector



A: Coomassie®-stained gel of β-gal produced from pET151 before (lane 1) and after (lane 2) cleavage with TEV protease
B: Immunoblot analysis of the same β-gal preparation using an anti V5-HRP antibody for detection.
Lane 1: uncleaved β-gal
Lane 2: β-gal treated overnight with TEV

Contents and Storage:

Each pET Directional TOPO® Expression Kit contains three boxes. The Directional TOPO® Expression box contains 200 ng of linearized, topoisomerase I-activated pET100/D-TOPO®, pET101/D-TOPO®, pET102/D-TOPO®, pET151/D-TOPO®, or pET200/D-TOPO® vector; sterile water; dNTPs; 10X PCR Buffer; salt solution; control template and primers; primers for sequencing or PCR screening; and an expression control. Store at -20°C. The One Shot® TOP10 box contains twenty-one 50-µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. Store at -80°C. The One Shot® BL21 Star™(DE3) box contains twenty-one 50-µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. Store at -80°C. Guaranteed stable for 6 months when properly stored.

Product	Reactions	Cat. no.
pET100 Directional TOPO® Expression Kit	20	K100-01
pET101 Directional TOPO® Expression Kit	20	K101-01
pET102 Directional TOPO® Expression Kit	20	K102-01
pET151 Directional TOPO® Expression Kit	20	K151-01
pET200 Directional TOPO® Expression Kit	20	K200-01



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