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 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.

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

<table>
<thead>
<tr>
<th>Vector</th>
<th>Fusion partner</th>
<th>Position</th>
<th>Tag</th>
<th>Cleavage protease</th>
<th>Antibiotic resistance</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>pET100/D-TOPO</td>
<td></td>
<td>N-term</td>
<td>Xpress™</td>
<td>EK</td>
<td>Amp</td>
<td>Cleavable detection and purification tag</td>
</tr>
<tr>
<td>pET101/D-TOPO</td>
<td></td>
<td>C-term</td>
<td>V5</td>
<td>N/A</td>
<td>Amp</td>
<td>Detection and purification tag</td>
</tr>
<tr>
<td>pET102/D-TOPO</td>
<td></td>
<td>N-term</td>
<td>thioredoxin</td>
<td>EK</td>
<td>Amp</td>
<td>Cleavable thioredoxin tag enhances protein translation and solubility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-term</td>
<td>V5</td>
<td>N/A</td>
<td>Amp</td>
<td>Detection and purification tag</td>
</tr>
<tr>
<td>pET151/D-TOPO</td>
<td></td>
<td>N-term</td>
<td>V5</td>
<td>TEV</td>
<td>Amp</td>
<td>Cleavable detection and purification tag</td>
</tr>
<tr>
<td>pET200/D-TOPO</td>
<td></td>
<td>N-term</td>
<td>Xpress™</td>
<td>EK</td>
<td>Kan</td>
<td>Cleavable detection and purification tag</td>
</tr>
</tbody>
</table>

Figure 1 - pET Directional TOPO® vectors
With the Directional TOPO®-adapted pET vectors you can expect the same proven expression achieved with the original pET vectors. Figures 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®.

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®, pET111/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® 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. 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. 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
PET111 Directional TOPO® Expression Kit 20 K111-01
PET200 Directional TOPO® Expression Kit 20 K200-01

The lacZ gene was directionally TOPO® Cloned into pET100/D-TOPO®, pET101/D-TOPO®, pET102/D-TOPO®, pET111/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 2 - Expression in Directional TOPO®-adapted pET vectors

Lane 1 and 2: pET101/D/lacZ
Lane 3 and 4: pET100/D/lacZ
Lane 5 and 6: pET102/D/lacZ
U = Uninduced  I = Induced

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