MultiShot™ TOP10 Chemically Competent E. coli

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Description
The MultiShot™ TOP10 Chemically Competent E. coli Kit is chemically competent TOP10 E. coli packaged in five 96-well plates to simplify high throughput bacterial transformation. After addition of DNA, cells can be transformed by heat-shock at 42°C using a heat block or thermocycler.

Contents
Each MultiShot™ Kit contains the following reagents. Each kit contains five 96-well plates of frozen competent cells.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Composition</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC medium</td>
<td>2% Tryptone; 0.5% Yeast Extract; 10 mM NaCl; 2.5 mM KCl; 10 mM MgCl2; 10 mM MgSO4; 20 mM glucose</td>
<td>5 x 15 mL</td>
</tr>
<tr>
<td>TOP10 cells</td>
<td>—</td>
<td>15 μL per well</td>
</tr>
<tr>
<td>pUC19</td>
<td>10 pg/μL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8</td>
<td>50 μL (500 pg)</td>
</tr>
</tbody>
</table>

Genotype
F- mcrΔ (nrr-lacIΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (Str8) endA1 nupG

Shipping and Storage
Each MultiShot™ Kit is shipped on dry ice. Upon receipt, store the kit at −85°C to −68°C.

Important!
Do not transform chemically competent cells by electroporation. The salt content of the buffer will cause arcing and kill the cells.

Introduction
The following procedure describes how we qualify the MultiShot™ Kit. This information is provided to help you determine how to best use the MultiShot™ Kit for your own applications.

Before Beginning
- Chill a 96-well metal heating block on ice until the block is cold.
- Bring the vial of SOC to room temperature.
- Pre-heat a heat block or thermocycler containing a 96-well metal block to 42°C.

Note: You can also use a water bath, but be careful not to contaminate the cells.
- If you are using a thermocycler, program the machine to hold the temperature at 42°C.

Procedure
1. Remove a MultiShot™ plate from the freezer and place it in the chilled metal block. Cells should thaw within 30 seconds.
2. Carefully remove the aluminum foil seal.
3. Use a multi-channel pipet to add 2 μL DNA (2 pg–20 ng) to the wells. Keep the volume around 2 μL for uniform results.

Note: You may need to dilute ligation reactions and TOPO® Cloning reactions with sterile water or TE buffer to prevent excess colonies.
4. Cover the cells with the supplied plastic lid and incubate the cells and DNA in the chilled block for 20 minutes.
5. Transfer the cell plate to either the pre-warmed heat block or the thermocycler, and heat-shock the plate for 30 seconds at 42°C.
6. Transfer the cell plate back to the chilled block and allow the plate to cool for 1 minute.
7. Remove the plastic lid and add 90 μL SOC to each well.
8. Cover and incubate the plate at 37°C for 1 hour. It is not necessary to shake the plate.
9. Plate the appropriate volume from each well. See the next column for examples and expected number of colonies.

Plating Volumes and Expected Results
The following table describes the type of DNA, amount transformed into MultiShot™ chemically competent cells, the volume plated, and the number of colonies.

<table>
<thead>
<tr>
<th>DNA</th>
<th>Type</th>
<th>Amount Transformed</th>
<th>Volume Plated</th>
<th>No. of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC19</td>
<td>Supercoiled 2.5 pg vector</td>
<td>10 μL</td>
<td>100–300</td>
<td></td>
</tr>
<tr>
<td>pCR2.1 plus PCR-amplified</td>
<td>TA ligation</td>
<td>200 pg vector +</td>
<td>Entire volume</td>
<td>100–400</td>
</tr>
<tr>
<td>750 bp insert</td>
<td></td>
<td>32 pg insert (diluted 1:50 from original ligation reaction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR™XL-TOPO® plus PCR-</td>
<td>TOPO® Cloning reaction</td>
<td>~3 ng vector +</td>
<td>Entire volume</td>
<td>100–200</td>
</tr>
<tr>
<td>amplified 7 kb insert</td>
<td></td>
<td>14 ng insert (undiluted)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Safety Data Sheets (SDSs)
Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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