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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.

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

Genotype

F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

Shipping and Storage

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

Note: You may store the SOC medium at 2°C to 8°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.

Note: We use pUC19 to qualify the kit. Transformation efficiency should be > 1 × 108 cfu/µg and yield 100–300 colonies per plate. Variability should be no more than 5-fold between wells.

DNA	Type	Amount Transformed	Volume Plated	No. of Colonies
pUC19	Supercoiled	2.5 pg vector	10 µL	100–300
pCR™2.1 plus PCR-amplified 750 bp insert	TA ligation	200 pg vector + 32 pg insert (diluted 1:50 from original ligation reaction)	Entire volume (~110 µL)	100–400
pCR™XL-TOPO® plus PCR-amplified 7 kb insert	TOPO® Cloning reaction	~3 ng vector + 14 ng insert (undiluted)	Entire volume (~110 µL)	100–200

Safety Data Sheets (SDSs)

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

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