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Catalog Number 200324
Product Name 96 Pack Gold Competent Cells
Expiration Date 2020-12-30
Quantity 4 Plates
Certified By Todd Parsons
Quality Controlled By Matt Huffman
Shipping Conditions Shipped on dry ice.

Materials Provided
96-Pack Gold competent cells, 4 × 96-well plates (15 μl/well)
pUC18 control plasmid (0.1 ng/μl in TE buffer), 10 μl
Tape, 12

Storage Conditions
Place cells at the bottom of a ~80°C freezer directly from the shipping container. Do not store in liquid nitrogen. Competent cells are sensitive to small temperature changes. Transferring tubes between freezers may result in a loss of efficiency.

Additional Materials Required
96-well thermal block.
Temperature cycler, water bath, or additional 96-well thermal block.

Guaranteed Efficiency
≥1 × 10^6 cfu/μg pUC18 DNA

Test Conditions
Transformations are performed both with and without pUC18 plasmid DNA, following the protocol outlined below. Following transformation, 20-μl samples of the culture are plated in duplicate on LB agar plates with 100 μg/ml ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Antibiotic Resistance
96-Pack Gold competent cells are tetracycline and chloramphenicol resistant.

Genotype and Background
TetA (mcrA)183 Δ(mcrCB-hsdSMR-mrr1)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lacY1 [F’ proAB lac(+)]ZAM15 Tn10 (Tet’ Amy Cam’). (Genes listed signify mutant alleles. Genes on the F’ episome, however, are wild-type unless indicated otherwise.)

96-Pack Gold competent cells are formatted for high-throughput cloning. Each plate contains 96 individual transformations for quick cloning of many constructs at once. 96-Pack Gold competent cells feature the XL10-Gold strain to give high transformation efficiency, especially for large and ligated DNA molecules. These cells also provide large colonies that grow quickly. 96-Pack Gold competent cells are ideal for constructing plasmid DNA libraries because using these cells decreases size bias and produces larger, more complex plasmid libraries. The XL10-Gold strain is deficient in all known restriction systems [Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr1)173]. The strain is endonuclease deficient (endA), greatly improving the quality of miniprep DNA, and recombination deficient (recA), helping to ensure insert stability. The lacZ(+)/ZAM15 gene on the F’ episome allows blue-white screening for recombinant plasmids.

Transformation Protocol
Preparation
1. Prepare SOC medium immediately before beginning the protocol (see Preparation of Media and Reagents).
2. Prepare for the heat pulse by doing one of the following:
   - (A) program a temperature cycler with a 96-well block to hold the temperature at 42°C and preheat the temperature cycler;
   - (B) preheat a 96-well heating block to 42°C, or
   - (C) preheat a water bath to 42°C. (Be careful to avoid cell contamination while heat-pulsing the transformation reaction in a water bath.)
3. Place a metal 96-well thermal block on ice to chill the block.

Protocol
1. Thaw the competent cells in a 96-well plate by placing the plate in a chilled metal 96-well block. The cells should thaw within 30 seconds.
2. Carefully remove the aluminum foil seal from the plate.
3. Using a multichannel pipettor, add 1 μl of DNA (1 pg – 20 ng) to each well. For uniform results, keep the volume near 1 μl. For a control, dilute the 0.1 ng/μl pUC18 DNA control plasmid 1:100 in high-quality water. Add 1 μl of the 1 pg/μl pUC18 DNA to each control well.
4. Seal the plate with tape.
5. Incubate the plate of cells and DNA in the chilled block for 20 minutes.
6. Heat-shock the cells for 20 seconds at 42°C by transferring the plate to a prewarmed temperature cycler, thermal block, or water bath. The duration of the heat pulse is critical for obtaining the highest transformation efficiency.
7. Transfer the plate back to the chilled block and allow the plate to cool for 1 minute.
8. Add 85 μl of SOC medium to each well.
9. Incubate the plate at 37°C for 1 hour. Shaking is not necessary.
10. Before plating, gently mix the cell suspensions by pipetting as cells may have settled to the bottom of the wells. Plate 10–100 μl of the suspensions on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired). For the pUC18 control transformation, plate 20 μl of the transformation on LB-ampicillin agar plates.
11. Incubate the plates at 37°C overnight. See Blue-White Color Screening, for color screening guidelines.
12. For the pUC18 control, expect 50–300 colonies (≥1 × 10^6 cfu/μg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA.