Made in USA

****Please note the new heat-shock temperature and duration****
See the transformation protocol for details

Catalog Number	**230350**
Product Name	**SoloPack Gold Supercompetent Cells**
Materials Provided	SoloPack Gold supercompetent cells, 15 single-tube transformations (50 µl each) pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 µl
Certified By	****
Quality Controlled By	****
Shipping Conditions	Shipped on dry ice.
Storage Conditions	Ultracompetent cells must be placed immediately at the bottom of a -80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen. Ultracompetent cells are sensitive to even small variations in temperature. Transferring tubes from one freezer to another may result in a loss of efficiency.
Guaranteed Efficiency	$\geq 1 \times 10^9$ cfu/µg pUC18 DNA
Test Conditions	Transformations are performed both with and without plasmid DNA using 50-µl aliquots of cells and 10 pg of pUC18 control DNA following the protocol outlined below. Following transformation, 5-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	**SoloPack Gold cells are tetracycline and chloramphenicol resistant.**
Genotype and Background	Tet^{r}Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Hte [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r) Amy Cam^r]. (Genes listed signify mutant alleles. Genes on the F′ episome, however, are wild-type unless indicated otherwise.)

SoloPack Gold supercompetent cells* are packaged in single-reaction aliquots, providing high performance and convenience. The single-tube transformation format eliminates the need to thaw, aliquot, and refreeze cells and allows for fewer pipetting steps because the entire transformation reaction occurs in the tube supplied. SoloPack Gold cells are deficient in all known restriction systems(McrA$^-$, McrCB$^-$, McrF$^-$, Mrr$^-$, HsdR$^-$), preventing the cleavage of methylated DNA. The strain is endonuclease deficient (*endA*), greatly improving the quality of miniprep DNA, and recombination deficient (*recA*), helping to ensure insert stability. The Hte phenotype increases the transformation efficiency of ligated and large supercoiled DNA and causes the production of large colonies. The *lacI*^{q}*ZΔM15* gene on the F′ episome allows blue-white screening for recombinant plasmids.

Transformation Protocol

1. Preheat NZY$^+$ broth or SOC broth to 42°C for use as the outgrowth medium in step 10.
   Note: *The optimized protocol uses NZY$^+$ broth for maximum transformation efficiency. Use of SOC results in slightly lower efficiencies. See* Critical Success Factors and Troubleshooting *(reverse page) for more information.*
2. Thaw two aliquots of SoloPack Gold cells on ice (one tube for each of the experimental and control transformations).
3. When the cells have thawed, swirl the tubes to gently mix the cells.
4. Add 0.01-50 ng of the experimental DNA to one tube of cells (see *Quantity and Volume of DNA*, on reverse page, for guidelines). Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other tube of cells.
5. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
6. Heat-pulse the tubes in a **42°C** water bath for 30 seconds. The temperature and duration of the heat pulse are critical for maximum efficiency. For consistent results, remove any ice trapped around the outside bottom of the tube.
7. Incubate the tubes on ice for 2 minutes.
8. Add 250 µl of preheated (42°C) NZY$^+$ broth or 250 µl of preheated SOC broth and incubate the tubes at 37°C for 1 hour with shaking at 225-250 rpm.
9. Plate ≤200 µl of the transformation mixture 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 5 µl of the transformation on LB-ampicillin agar plates.
10. Incubate the plates at 37°C overnight. If performing blue-white color screening, incubate the plates at 37°C for at least 17 hours to allow color development (color can be enhanced by subsequent incubation of the plates for 2 hours at 4°C).
11. For the pUC18 control, expect 167 colonies ($\geq 1 \times 10^9$ cfu/µg pUC18 DNA).

Blue-White Color Screening

Blue-white color screening for recombinant plasmids is available when transforming this host strain (containing the *lacI*^{q}ZΔ*M15* gene on the F′ episome) with a plasmid that provides α-complementation (e.g. Stratagene pBluescript II). When lacZ expression is induced by IPTG in the presence of the chromogenic substrate X-gal, colonies containing plasmids with inserts will be white, while colonies containing plasmids without inserts will be blue. If an insert is suspected to be toxic, plate the cells on media without X-gal and IPTG. Color screening will be eliminated, but lower levels of the potentially toxic protein will be expressed in the absence of IPTG.

Critical Success Factors and Troubleshooting

Use of NZY$^+$ broth: For the highest transformation efficiencies (1×10^9 cfu/µg pUC18 DNA), use NZY$^+$ broth as the outgrowth medium.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl supercoiled pUC18 DNA per aliquot of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/µg) may be lower. The volume of the DNA solution added to the reaction may be increased to up to 5 µl, but the transformation efficiency may be reduced.

Heat Pulse Duration and Temperature: Optimal transformation efficiency is observed when cells are heat-pulsed at **42°C** for 30 seconds in the tubes provided. Efficiency decreases sharply when either the temperature or the duration of the heat pulse is altered.

Plating the Transformation Mixture: If plating <100 µl of cells, pipet the cells into a 200-µl pool of medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells.

Preparation of Media and Reagents

SOC Broth (per Liter)

Prepare SOB medium:
20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add dH_2O to a final volume of 1 liter and then autoclave
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

Prepare SOC medium immediately before use:
98 ml of SOB medium
2 ml of filter-sterilized 20% (w/v) glucose

NZY$^+$ Broth (per Liter)

10 g of NZ amine (casein hydrolysate)
5 g of yeast extract
5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Adjust to pH 7.5 using NaOH and then autoclave
Add the following filer-sterilized supplements prior to use:
12.5 ml of 1 M $MgCl_2$
12.5 ml of 1 M $MgSO_4$
20 ml of 20% (w/v) glucose

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH and then autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB-Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved and cooled to 55°C
Add 10 ml of 10 mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

Plates for Blue-White Color Screening

Prepare the LB agar and when adding the antibiotic, also add 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to a final concentration of 80 µg/ml [prepared in dimethylformamide (DMF)] and isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile water). Alternatively, 100 µl of 10 mM IPTG and 100 µl of 2% X-gal may be spread on solidified LB agar plates 30 minutes prior to plating the transformations. (For consistent color development across the plate, pipet the X-gal and the IPTG into a 100-µl pool of SOC medium and then spread the mixture across the plate. Do not mix the IPTG and the X-gal before pipetting them into the pool of SOC medium because these chemicals may precipitate.)

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Endnotes

*U.S. Patent Nos. 5,512,468 and 5,707,841 and patents pending and equivalent foreign patents.

For in vitro use only. This certificate is a declaration of analysis at the time of manufacture.