Made in USA

Catalog Number **200150**

Product Name **XL2-Blue Ultracompetent Cells**

Materials Provided
XL2-Blue ultracompetent cells (blue tubes), 10 × 100 µl
pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 µl
β-Mercaptoethanol (1.22 M), 25 µl

Certified By Todd Parsons

Quality Controlled By Tricia Molina

Shipping Conditions Shipped on dry ice.

Storage Conditions Competent 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. Competent 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 ≥5.0×10^9 cfu/µg pUC18 DNA

Test Conditions Transformations are performed both with and without plasmid DNA using 100-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.

Genotype and Background *endA1 supE44 thi-1 hsdR17 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r) Amy Cam^r]. (Genes listed signify mutant alleles. Genes on the F′ episome, however, are wild-type unless indicated otherwise.)

XL2-Blue ultracompetent cells* are high-efficiency derivatives of Stratagene's XL1-Blue supercompetent cells. Using ultracompetent cell technology, Stratagene has achieved transformation efficiencies greater than 5×10^9 cfu/µg of pUC18 DNA, making these cells an ideal choice for high-efficiency cloning. XL2-Blue cells are endonuclease (*endA*), and recombination (*recA*) deficient. The *hsdR* mutation prevents cleavage of cloned DNA by the *Eco*K endonuclease system. The *lacI*^{q}*ZΔM15* gene on the F′ episome allows blue-white screening for recombinant plasmids.

Antibiotic Resistance **XL2-Blue cells are tetracycline and chloramphenicol resistant.**

Transformation Protocol

1. Pre-chill two 14-ml BD Falcon polypropylene round-bottom tubes on ice. (One tube is for the experimental transformation and one tube is for the pUC18 control.) Preheat NZY$^+$ medium to 42°C.
2. Thaw the cells on ice. When thawed, gently mix and aliquot 100 µl of cells into each of the two pre-chilled tubes.
3. Add 2 µl of the β-ME provided with this kit to each aliquot of cells.
4. Swirl the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
5. Add 0.1-50 ng of the experimental DNA to one aliquot of cells, in a 1-µl volume for maximum efficiency. Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other aliquot of cells.
6. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is critical.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated (42°C) NZY$^+$ broth and incubate the tubes at 37°C for 1 hour with shaking at 225-250 rpm.
10. 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 mixture on LB-ampicillin agar plates.
11. 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).
12. For the pUC18 control, expect 250 colonies (≥5×10^9 cfu/µg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA, with larger and non-supercoiled DNA producing fewer colonies.

Blue-White Color Screening Blue-white color screening for recombinant plasmids is available when transforming this host strain (containing the *lacI*^{q}*ZΔM15* gene on the F′ episome) with a plasmid that provides α-complementation (e.g. the Stratagene pBluescript II vector). 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 14-ml BD Falcon polypropylene round-bottom tubes: It is important that 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) are used for the transformation protocol, since other tubes may be degraded by β-mercaptoethanol. In addition, the duration of the heat pulse has been optimized using these tubes.

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into pre-chilled tubes. It is also important to use 100 µl of cells per transformation. Decreasing the volume will reduce efficiency.

Use of β-Mercaptoethanol (β-ME): β-ME has been shown to increase transformation efficiency. The β-ME mixture provided is diluted and ready to use. Stratagene cannot guarantee results with β-ME from other sources.

Use of NZY$^+$ Broth: Transformation of the supplied ultracompetent cells has been optimized using NZY$^+$ as the medium for outgrowth following the heat pulse. Substitution with another outgrowth medium may result in a loss of efficiency.

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 100 µl of cells. When transforming a ligation mixture, add 2 µl of the ligation mixture per 100 µl 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 10% of the reaction volume, 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. Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >40 seconds. Do not exceed 42°C.

Plating the Transformation Mixture: If plating <100 µl of cells, pipet the cells into a 200-µl pool of NZY$^+$ 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. If desired, cells may be concentrated prior to plating by centrifugation at 1000 rpm for 10 minutes followed by resuspension in 200 µl of NZY$^+$ medium.

Preparation of Media and Reagents

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 (or 10 ml of 2 M 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.)

Limited Product Warranty

Endnotes

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

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