

**Made in USA**

**Catalog Number**

**200230**

**Product Name**

**XL1-Blue MRF<sup>+</sup> Supercompetent Cells**

**Materials Provided**

XL1-Blue MRF<sup>+</sup> supercompetent cells (yellow tubes), 5 × 200 µl  
pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 µl  
β-Mercaptoethanol (1.42 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**

≥1.0 × 10<sup>9</sup> cfu/µg pUC18 DNA

**Test Conditions**

Transformations are performed both with and without plasmid DNA using 100-µl aliquots of cells and 100 pg of pUC18 control DNA following the protocol outlined below. Following transformation, 2.5-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**

*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F' *proAB lacI<sup>q</sup>ZAM15 Tn10* (Tet<sup>r</sup>)]. (Genes listed signify mutant alleles. Genes on the F' episome, however, are wild-type unless indicated otherwise.)

The XL1-Blue MRF<sup>-</sup> (Minus Restriction) strain is a restriction minus (McrA<sup>-</sup>, McrCB<sup>-</sup>, McrF<sup>-</sup>, Mrr<sup>-</sup>, HsdR<sup>-</sup>) derivative of Stratagene's XL1-Blue strain. XL1-Blue MRF<sup>-</sup> cells are deficient in all known restriction systems [*Δ(mcrA)183*, *Δ(mcrCB-hsdSMR-mrr)173*], and are endonuclease (*endA*), and recombination (*recA*) deficient. The *hsdR* mutation prevents the cleavage of cloned DNA by the *EcoK* endonuclease system, and the *recA* mutation helps ensure insert stability. The *endA* mutation greatly improves the quality of miniprep DNA. The *lacI<sup>q</sup>ZAM15* gene on the F' episome allows blue-white screening for recombinant plasmids.

**Antibiotic Resistance**

**XL1-Blue MRF<sup>-</sup> cells are resistant to tetracycline.**

**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 SOC 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 1.7 µl of the β-mercaptoethanol 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 and add 1 µl of the pUC18 control DNA to the other aliquot. Swirl the tubes gently.
6. Incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 45 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) SOC medium 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 2.5 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 (≥1 × 10<sup>9</sup> 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<sup>q</sup>ZAM15* 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  $\beta$ -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 the volume of cells indicated in step 2 of the *Transformation Protocol*. Decreasing the volume will reduce efficiency.

**Use of  $\beta$ -Mercaptoethanol ( $\beta$ -ME):**  $\beta$ -ME has been shown to increase transformation efficiency. The  $\beta$ -ME mixture provided is diluted and ready to use. A fresh 1:10 dilution (from a 14.2 M stock) may be used; however, Stratagene cannot guarantee results with  $\beta$ -ME from other sources.

**Quantity and Volume of DNA:** The greatest efficiency is obtained from the transformation of 1  $\mu$ l of 0.1 ng/ $\mu$ l supercoiled pUC18 DNA per 100  $\mu$ l of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/ $\mu$ g) 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 45-50 seconds. Efficiency decreases sharply when cells are heat-pulsed for <45 seconds or for >60 seconds. Do not exceed 42°C.

**Plating the Transformation Mixture:** If plating <100  $\mu$ l of cells, pipet the cells into a 200- $\mu$ l pool of SOC medium and then spread the mixture with a sterile spreader. If plating  $\geq$ 100  $\mu$ l, 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  $\mu$ l of SOC medium.

## Preparation of Media and Reagents

### SOB Medium (per Liter)

20.0 g of tryptone

5.0 g of yeast extract

0.5 g of NaCl

Add deionized H<sub>2</sub>O to a final volume of 1 liter and then autoclave

Add 10 ml of filter-sterilized 1 M MgCl<sub>2</sub> and 10 ml of filter-sterilized 1 M MgSO<sub>4</sub> prior to use

### SOC Medium (per 100 ml)

**Prepare immediately before use**

2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose

SOB medium (autoclaved) to a final volume of 100 ml

### LB Agar (per Liter)

10 g of NaCl

10 g of tryptone

5 g of yeast extract

20 g of agar

Add deionized H<sub>2</sub>O 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- $\beta$ -D-galactopyranoside (X-gal) to a final concentration of 80  $\mu$ g/ml [prepared in dimethylformamide (DMF)] and isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile dH<sub>2</sub>O). Alternatively, 100  $\mu$ l of 10 mM IPTG and 100  $\mu$ l 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- $\mu$ l 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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