

SS320 (MC1061 F') super Electrocompetent Cells

Introduction:

SS320 (MC1061 F') Electrocompetent Cells deliver $\geq 4 \times 10^{10}$ cfu/ μ g of DNA and are particularly useful for phage display protein expression. SS320, also known as MC1061 F' cells, are also suitable for M13 phage work, general cloning, blue/white screening and protein expression.

Specifications:

Competence:	Electroporation Competent
Application:	Phage Display
Organism:	E. coli

Applications:

Phage display library construction and screening

Antibody epitope mapping

Peptide ligand identification

Protein-Protein interaction identification

Directed evolution of proteins

Find tumor antigens, candidates for therapeutic antibodies, enzyme inhibitors, receptor agonists, etc.

Features:

Transformation Efficiency $\geq 4 \times 10^{10}$ cfu/ μ g pUC19.

Control DNA included.

Genotype:

[F'proA+B+ lacIqlacZ Δ M15 Tn10 (tetr)] hsdR mcrB araD139 Δ (araABC- leu)7679 Δ lacX74 galUgalK rpsL thi

Storage conditions

Electrocompetent cells require storage at -80 °C

Usage Guidelines

Preparation for transformation

Transformation is carried out in a 0.1 cm gap cuvette using 25 μ L of cells. Optimal settings for electroporation are listed in Table 1 below. Typical time constants are 4.5 to 5.2 msec.

Condition	Optimal Setting
Cuvette gap	1.0 mm
Voltage	1,800 V
Capacitance	10 μ F
Impedance	600 Ohms

Suggested Electroporation Systems:

Bio-Rad Micro Pulser #165-2100; Bio-Rad E. coli Pulser #165-2102; Bio-Rad Gene Pulser II #165- 2105; BTX ECM630 Electroporation System; Eppendorf Electroporator 2510.

Transformation protocol

To ensure successful transformation results, the following precautions must be taken:

- Ligation reactions must be heat killed at 70 °C for 15 minutes before transformation. The ligation reaction can be used directly after heat inactivation, without purification of the ligation products.

- DNA samples must be purified and dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in loss of the cells and DNA.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed on ice before use.

1. Have 2*TY Miller and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction).
2. Place electroporation cuvettes and microcentrifuge tubes on ice (one cuvette and one microfuge tube for each transformation reaction).
3. Remove Electrocompetent Cells from the -80 °C freezer and place on wet ice until they thaw completely (10-15 minutes).
4. When the cells are thawed, mix them by tapping gently. Aliquot 25µL of cells into the chilled microcentrifuge tubes on ice.
5. Add 1µL of the heat-denatured ligation reaction to the 25µL of cells on ice. Failure to heat-inactivate the ligation reaction will prevent transformation. Stir briefly with pipette tip; do not pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2µL of ligation mix may cause electrical arcing during electroporation.

For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.

6. Carefully pipet 25µL of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
7. Within 10 seconds of the pulse, add 975µL of 2*YT Miller to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
8. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Spread up to 100µL of transformed cells on 2*YT agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in 2*YT culture medium.

Media recipes

2*TY agar plates

Per litre:

- 16 g tryptone
- 10 g yeast extract
- 5 g NaCl
- 15 g agar

Medium for growth of transformants

2*YT

Per litre:

- 16 g tryptone
- 10 g yeast extract
- 5 g NaCl

Add all components to deionised water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

Technical support and product guarantee

Alpvhhs is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

If you require any further support, please do not hesitate to contact our Technical Support Team: service@alpvhhs.com.

Product guarantee: Alpvhhs guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.