Acella™ Electrocompetent Cells

Product	Cat No.	Transformations
Acella™ Electrocompetent Cells	42649	30

Description

Acella™ Electrocompetent Cells are an electrocompetent ∆*end*A ∆*rec*A derivative of BL21 (DE3) cells. Acella™ Electrocompetent Cells have been manufactured using a proprietary technology rendering the cells highly efficient for DNA uptake when used in an electroporation device. To utilize the cells at their highest efficiency, a recommended transformation protocol is included with each kit.

The complete deletions of the *endA* and *recA* genes eliminate plasmid recombination and provide excellent yield and quality plasmid DNA, making this strain ideal for direct cloning and protein expression. By cloning directly in the Acella™ strain, you save at least two days of work normally spent on subcloning procedures and you eliminate the need of additional highly efficient competent cells for the cloning procedure.

Cloning and expression steps can be combined by directly transforming the Acella™ Electrocompetent Cells with the ligation products using the enclosed protocol. Transformed cells should be plated on selective media and incubated at 37°C overnight. Since Acella™ Electrocompetent Cells are fast growing, miniprep cultures can be grown for 4-5 hours and plasmids can be analyzed the day after the transformation, saving one extra day. Positive clones can then be grown directly for protein expression.

Like all BL21 cells, Acella™ Electrocompetent Cells lack the Lon and OmpT proteases, promoting stability of recombinant proteins. Acella™ Electrocompetent Cells carry a copy of the T7 RNA polymerase and are ideal for expression of T7 promoter-driven constructs.

Acella™ Electrocompetent Cells are provided in tubes. All kits include a test plasmid for quality control purposes. Each tube contains pre-dispensed cells in 100µl aliquots, enough for 5 transformations using the recommended protocol.

Full processing time for one tube (including recovery) is approximately one hour. Edge BioSystems guarantees transformation efficiencies of Acella™ Electrocompetent Cells ≥ 10^{10} cfu/µg pUC19.

Genotype

Acella™ Electrocompetent Cells: F^- *omp*T *hsd*$S_B(r_B^- m_B^-)$ *gal dcm* (DE3) ∆*endA* ∆*recA*

Kit Components	42649
Acella™ Electrocompetent Cells	6 tubes
pUC19 Supercoiled DNA, 100ng/ml	1 tube

Quality Control

Each lot is tested to assure high transformation efficiency using 10 pg pUC19 supercoiled DNA and the recommended protocol. Transformation efficiency will be ≥10^{10} cfu/µg pUC19 under these conditions.

Equipment and Materials Not Provided

1. SOC medium for recovery: 20g/l tryptone, 5g/l yeast extract, 10mM NaCl, 2.5mM KCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20mM glucose ($MgCl_2$, $MgSO_4$ and glucose should be added after autoclaving).
2. An orbital shaker capable of 37°C and 250 rpm.
3. An electroporation device with electroporation cuvettes.
4. 14ml round-bottom culture tubes (one tube per transformation).
5. LB-agar selective plates or selective liquid media.
6. Pipettors.

Storage Conditions

Acella™ Electrocompetent Cells should be stored in a -80ºC freezer. Please note that competent cells are very sensitive to cycles of freezing and thawing and should not be exposed to temperature variations.

Recommended Protocol

1. Place tubes on ice and for approximately 5-10 minutes to thaw.
2. Chill on ice one microcentrifuge tube and one electroporation cuvette for each sample.

Warning: This product is intended for **research use only**. It is not to be used for diagnostic purposes in humans or animals.

3. Mix thawed cells by gently tapping the bottom of the tube. Aliquot 20-40µl of cells into each chilled microcentrifuge tube. Control transformation: Use 20µl of Acella™ Electrocompetent Cells. Excess cells can be re-frozen in a dry ice/ethanol bath and returned to the -80°C freezer.

   Note: **Re-frozen cells will have reduced transformation efficiency.**

4. Pipette the DNA to be transformed (1-5µl) to the bottom of the tube containing cells and mix by gently tapping the bottom of the tube. Control transformation: Dilute pUC19 supercoiled DNA 1:10 with dH_2O, then add 1µl of the diluted pUC19 supercoiled DNA to the control transformation tube. Discard the remaining diluted pUC19 supercoiled DNA after use.

   Note: **Do not mix by pipetting up and down since that will lower the transformation efficiency. Mix and electroporate only one sample at a time.**

5. Immediately transfer cell/DNA mix into a pre-chilled electroporation cuvette.

   Note: **Do not introduce bubbles into the sample.**

6. Electroporate samples.

   - For the BioRad GenePulser® II, use the following conditions: 1.4kV, 200Ω, 25µF.

7. Add one ml SOC medium to the cuvette and transfer the total mixture to a 14 ml round-bottom tube.

8. Place the tubes in a shaking incubator and then shake at 250 rpm at 37°C for one hour.

9. Dilute samples as necessary with SOC medium. Control reaction: Dilute the pUC19 control reaction 1:100 with SOC medium.

10. Plate 100-200µl of sample onto pre-warmed LB-agar selective plates or inoculate into selective liquid medium. Control transformation: Plate 50µl on LB-ampicillin agar plates and expect 50 colonies ($>10^{10}$ cfu/µg pUC19).

Additional Notes

a. Transformation efficiencies for ligation mixtures will be 10-100 fold lower than pUC19 supercoiled DNA ligations. It is important to remove salts and buffers from any samples prior to electrotransformation since they will inhibit electroporation. Ligation reactions should be ethanol-precipitated or diluted before electrotransformation. Ligations can be diluted 1:5 with dH_2O. Excess salt, buffer or DNA can cause arcing.

b. Using an electroporator other than the BioRad GenePulser® II may require setting adjustments in order to obtain the optimal efficiency for that specific model.

c. Calculating transformation efficiency

   [(cfu on control plate) / (pg of supercoiled pUC19)] x (10^6 pg / µg) x (final dilution) = cfu / µg DNA

   (Note: cfu = colony forming units)

   For example, if the control reaction plate has 50 colonies when 50µl of diluted reaction is plated then,

   [(50 cfu) / (10 pg)] x (10^6 pg) / (µg) x (1 ml) / (0.05 ml plated) X 10^2 = 1.0×10^{10} cfu/µg pUC19 DNA

Special Note

Acella™ Electrocompetent Cells are based on the T7 expression system. This technology was developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy. Consequently, U.S. patents assigned to Brookhaven Science Associates (BSA) protect this technology. These materials are to be used by noncommercial entities for research purposes only. Commercial entities require a license from BSA. You may refuse these cells by returning the enclosed materials unused. To obtain information about licensing, please contact the Office of Intellectual Property and Partnerships, Brookhaven National Laboratory, Building 475D, Upton, NY 11973 (telephone: 631-344-7134 or fax: 631-344-3729).

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