



Frequently Asked Questions for
Acella™ Electrocompetent Cells

About Your Acella™ Electrocompetent Cells

1. How can we easily distinguish strain types when looking at your box?

Different strains are clearly labeled as such on the box. Please note that tubes of cells are not individually labeled.

2. How are the *E. coli* strains derived?

These strains are derivatives from the standard BL21 (DE3) strains initially developed by the Brookhaven National Laboratories. We deleted the *endA* and *recA* genes without introducing additional mutations or antibiotic resistance. The only difference between the standard BL21 (DE3) strains developed by the Brookhaven National Laboratories and our Acella™ strain is the complete deletion of those two genes.

3. What is the genotype and relative advantage of the electrocompetent version of Acella™ Electrocompetent Cells?

Genotype: F⁻ *ompT hsdS_B(r_B⁻m_B⁻) gal dcm* (DE3) Δ *endA* Δ *recA*

Genotype	Advantage
DE3	A lambda derivative bacteriophage that carries the gene for T7 RNA polymerase under the control of the <i>lacUV5</i> promoter (inducible by IPTG)
<i>ompT</i>	Deficiency in the <i>ompT</i> protease, which results in higher yields of intact recombinant proteins
<i>hsdS_B (r_B⁻ m_B⁻)</i>	Allows cloning of DNA without cleavage by endogenous restriction endonucleases
Δ <i>endA</i>	Complete deletion of <i>endA</i> gene (DNA specific endonuclease I) shown to improve yield and quality of DNA from plasmid minipreps.
Δ <i>recA</i>	Complete deletion of <i>recA</i> gene (gene central to general recombination and DNA repair) shown to eliminate general recombination and render bacteria sensitive to UV light.

4. For what purpose(s) is your Acella™ Electrocompetent Cells bacterial strain used?

Their high transformation efficiency and Δ *endA* Δ *recA* genotype make them ideal as a standard cloning strain with high plasmid yield and their BL21 background makes them a fast growing strain. These cells are ideal for high-level protein expression since they lack both *ompT* and *lon* proteases. Acella™ Electrocompetent Cells are particularly effective in directly transforming cDNA libraries with an expression vector. Acella™ Electrocompetent Cells can be used for cloning and expression purposes in just one transformation step and they have the advantage of fast growth, making it possible to isolate miniprep plasmid DNA after only three hours of culture. All of these features make it possible to save a total of two to three days of work and eliminate the need for additional cloning strains and reagents.

5. What efficiencies do your Acella™ Electrocompetent Cells attain?

Edge BioSystems guarantees >10¹⁰ colonies / μ g pUC19. As with any other electrocompetent cell, the transformation efficiency may decrease with larger plasmids or ligated DNA.

6. What are the advantages and disadvantages of using *E. coli* for protein expression?

Many recombinant proteins can be cloned and expressed in *E. coli*. The use of *E. coli* for protein expression is well documented for its advantages of low cost, easy transformation and fermentation, and high protein yields. However,



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solubility may be an issue, since some proteins are insoluble and aggregate in inclusion bodies. Some proteins require post-translational modifications in order to be completely functional and *E. coli* cells do not perform these modifications. It is important to note that the following factors can affect expression levels and/or solubility: growing temperature, concentration of inducer (IPTG), host strain, protein size and structure, and toxicity of the protein.

7. Can I use my Acella™ Electrocompetent Cells for non-research purposes?

Edge BioSystems provides these materials for research purposes only. Acella™ Electrocompetent Cells are based on the T7 expression system. This is technology 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).

8. How do Acella™ Electrocompetent Cells best fit my needs?

The following chart can be used as a general guideline:

Electrocompetent Cell Strain	Description	Application
Acella™	(DE3) indicates that the host is a lysogen of λ DE3 and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the <i>lacUV5</i> promoter.	Recombinant protein production non-toxic to <i>E. coli</i> , high quality plasmid preparation with short culture time (3 hours) for plasmid miniprep.

Protein Expression

1. Is it possible to directly clone, propagate and express into Acella™ Electrocompetent Cells?

Yes. Acella™ Electrocompetent Cells have transformation efficiencies exceeding 10^{10} , making it possible to eliminate the intermediate step of cloning into a different strain of *E. coli*, purifying the DNA and then transforming into a BL21 strain with a lower transformation efficiency. You are thereby able to directly transform cDNA libraries into Acella™ Electrocompetent Cells and readily purify the DNA. In fact, high transformation efficiencies make Acella™ suitable for applications that include a need for increased plasmid yield and quality and generation of large plasmid libraries. The complete deletion of *endA* and *recA* genes makes this strain ideal for cloning purposes and DNA isolation. Since Acella™ Electrocompetent Cells are a fast growing strain, miniprep cultures can be grown for 3-4 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.

2. Are Acella™ Electrocompetent Cells good for protein expression?

Yes. With the exception of the added $\Delta endA \Delta recA$ genotype, the Acella™ Electrocompetent Cells are identical to standard BL21. BL21 cells naturally lack *ompT* and Lon proteases improving stability of synthesized proteins. BL21 (DE3) expresses T7 polymerase after induction with IPTG, therefore regulating the transcription of genes under the T7 promoter (for example, those in pET plasmids).

3. What proteins are the Acella™ Electrocompetent Cells good at expressing?

The expression properties of Acella™ Electrocompetent Cells are the same as the standard BL21. It is difficult to predict because protein levels as well as solubility will vary from protein to protein. In general, long proteins are much more difficult to express than shorter proteins. Also, human proteins that contain clusters of codons rarely used in *E. coli* may have a tendency to give lower yields and/or truncated products.



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4. How is expression controlled?

Our Acella™ Electrocompetent Cells are designed for high-level protein expression using T7 RNA polymerase-based expression systems. Acella™ pLysS Chemically Competent Cells provides tighter control for expression of toxic proteins. Tighter control is provided by strains carrying the pLysS plasmids encoding T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

5. How can we best express toxic proteins?

Proteins toxic to *E.coli* are better expressed in Acella™ Chemically Competent Cells containing the pLysS plasmid. The pLysS plasmid will express low levels of lysozyme that will bind to T7 polymerase, therefore inhibiting transcription. This will lower the basal expression of the protein during the pre-induction growth. If the protein is extremely toxic, then it is better expressed in a BL21 (DE3) pLysE strain or using a combination of a Acella™ Electrocompetent Cells and a T7 *lac* promoter.

Electroporation

1. What is the importance of field strength for electroporation?

Field strength is critical in electroporation. Field strength is usually expressed as kilovolts/centimeter (kV/cm), where kV is equal to the initial peak voltage and cm is equal to the size of the gap between the electrodes of the cuvette.

2. How can I prevent arcing when I electroporate bacterial cells?

Avoiding conductive ions is a good way to avoid arcing when electroporating bacteria. This can be achieved by lowering the ratio of DNA-to-cells in the sample. Always avoid air bubbles and condensation that can accumulate on the electroporation cuvette. Dilute or precipitate ligation reactions before electroporation, carefully following washing procedures to remove salts. For ligation reactions dilute at least 1:5 with water or TE, or use a proven method for desalting before electroporation. Do not use more than 5µl total volume of DNA with 20µl cells. Using only high-quality cuvettes can also reduce the chances of arcing.

3. Which cuvettes should be used when electroporating Acella™ Electrocompetent Cells?

Because field strength is critical for bacteria, the choice of cuvette is important. In order to achieve the proper pulse, we suggest 1mm cuvettes. Yeast and fungi require 2mm cuvettes, while 4mm cuvettes are ideally suited to mammalian and human cells.

4. When I pulled out my transformation plates from the incubator the next day, I had a lot of little colonies around big colonies. What are they?

These are satellite colonies. They are not transformants. Incubate your plates for less time (18 hours is good -- never more than 20), use more antibiotic, or use fresher plates to get rid of them.

5. How much DNA should I add to my cells for a transformation?

We recommend using 1µl DNA with 20µl cells for each transformation, larger size reactions can be used (up to 40µl in a 0.1cm gap cuvette) but the volume of DNA used should not be more than 5% of the cell volume.

Ligation

1. Does ligated DNA need to be diluted or desalted before adding it to Acella™ Electrocompetent Cells?

Yes, ligated DNA does require dilution or desalting prior to transformation. For ligation reactions dilute at least 1:5 with water or TE buffer, or use a proven method for desalting before electroporation.

2. How is transformation efficiency affected by the amount of DNA used during ligation?

The more DNA used, the more efficient the ligation will be. The ratio of vector to inserted DNA can be critical for obtaining high-efficiency ligations. A molar excess of insert to vector may yield higher efficiency ligations when



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subcloning inserts into plasmid vectors. However, an equal or greater ratio of vector to insert may be preferred when performing library construction into plasmid vectors. Before electroporation, the DNA should be diluted or precipitated as high DNA concentration in the electroporation can cause arcing.

Induction

1. Do higher IPTG concentrations yield better expression results?

The optimal concentration of IPTG may vary from protein to protein. In other words, a concentration that works well for one protein may be too high for another, therefore resulting in insolubilization. In general, 0.4mM IPTG provides full induction of genes under the T7 promoter and 1mM is recommended for full induction of genes under the T7-*lac* promoter.

2. What promoter do the Acella™ Electrocompetent Cells use?

Acella™ Electrocompetent Cells express the T7 polymerase under induction with IPTG. Plasmids containing the gene of interest under the control of a standard T7 promoter or a variant of it (for example, a T7-*lac* promoter) can be used with this system.

Freezing and Thawing

1. Can I reuse my competent cells once the tube has thawed?

No, freeze/thaw cycles will greatly lower transformation efficiencies. However, if you want to use a tube for several transformations, we recommend quickly refreezing any remaining cells on dry ice immediately after use. Return refrozen cells to -80°C for storage.

2. How should I store my competent cells when they arrive?

Upon receipt of your cell shipment, cells should immediately be placed at -80°C to ensure optimal activity. Competent cell stability in terms of transformation efficiency is guaranteed by Edge BioSystems for at least 12 months, when properly stored at -80°C.

Clone Yield Potential

1. Is it possible to predict the number of clones you will generate?

No, transformation efficiency, although proportional to the number of clones generated, is only one of many factors that will determine the number of clones after ligation of library construction. Other factors include: Amount of vector used, quality of DNA, size of DNA, method of generating DNA (i.e. PCR, miniprep, etc.), restriction enzyme used (blunt or cohesive ends), systems used to generate clones (i.e. recombination or conventional ligation) and others. Acella™ Electrocompetent Cells have been thoroughly controlled for quality and are guaranteed to exceed transformation efficiencies of 10^{10} colonies/ μ g with the pUC19 supercoiled DNA supplied.

Protein Yield Potential

1. Is it possible to predict protein yields by the strain?

No. Protein levels depend on many factors, including the protein that is expressed.

Quality Control

1. What quality control can be performed to test the transformation efficiency of the competent cells?

We calculate the transformation efficiency of our competent cells with 10pg supercoiled pUC19 and expect a transformation efficiency of at least 1×10^{10} colonies/ μ g pUC19 supercoiled DNA. Additionally the cells are tested with 10ng of pUC19 supercoiled DNA to insure that they can produce at least 1×10^8 colonies per 20 μ l electroporation reaction.



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Growth Conditions

1. How does the growth of Acella™ Electrocompetent Cells differ from other BL21 strains currently on the market?

Acella™ Electrocompetent Cells grow at the same rate in liquid medium and they grow slightly slower than standard BL21 cells in agar plates. Growth of Acella™ Chemically Competent Cells is several times faster than K-12 derivative cloning strains in liquid and in agar medium.

Transformation and Recovery

1. How important is SOC medium when using Acella™ Electrocompetent Cells?

An incubation of 45-60 minutes with SOC lets bacteria recover after transformation. Our transformation efficiencies are calculated using a standard 60-minute incubation with SOC. If SOC recovery is omitted, the transformation efficiency may decrease up to 10-fold for ampicillin-resistant plasmids and up to 100-fold for kanamycin-resistant plasmids. Incubation in other medium, different from SOC during recovery may result in lower transformation efficiencies.

2. What formulation of SOC medium works best with this application?

Standard SOC medium can be prepared as follows:

<u>Ingredients</u>	<u>Final Concentration</u>
Yeast Extract	5g/l
Tryptone	20g/l
Sodium Chloride	10mM
Potassium Chloride	2.5mM

Autoclave, then add:

Glucose	20mM
Magnesium Chloride	10mM
Magnesium Sulfate	10mM

3. Can I recover my cells in less than 1 hour (as indicated in the recommended protocol)?

When transforming with an ampicillin-based plasmid, 15-30 minutes of recovery will give about 70% of the standard efficiency. For kanamycin-based plasmids, 30 minutes of recovery will give about 50% of the standard efficiency, and 45 minutes recovery will give efficiency similar to 1 hour of recovery.

4. Can I add SOC directly to the 1.5ml tube of competent cells?

This does not directly apply since the cells are rinsed out of the cuvette with SOC before expression.

Transformation: Natural and Artificial

1. What does “transformation” really mean?

Transformation is the process by which naked DNA is introduced into cells. It also can happen naturally at a low frequency.

2. What exactly are “competent cells?”

These are cells that have been physically manipulated to increase their transformation efficiency.

3. What is “transformation efficiency?”

Transformation efficiency refers to the number of cells that are transformed by 1µg DNA, usually a supercoiled plasmid.



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4. How do I calculate the transformation efficiency?

$[(\text{cfu on control plate}) / (\text{pg of supercoiled pUC19})] \times (10^6 \text{ pg} / \mu\text{g}) \times (\text{final dilution}) = \text{cfu} / \mu\text{g DNA}$

(Note: cfu = colony forming units)

5. How are cells made competent?

Cells can be made competent for transformation chemically or by electroporation. Chemical competency usually involves treatment with divalent cations at low temperatures, followed by a quick cold-heat transfer during transformation. Electroporation involves the removal of salts that may cause “arcing” during the electrical shock. Both methods provide cells that can be frozen for storage.