Frequently Asked Questions for
Acella™ Chemically Competent Cells,
Acella™ pLysS Chemically Competent Cells

<u>About Your Acella™ Chemically Competent Cells and Acella™ pLysS Chemically Competent Cells</u>

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

Different strains are clearly labeled as such. While we do not label the aluminum seal affixed to the plate product, there are other distinguishing labels that make identification easy. Labeling the aluminum seal would prevent the piercing characteristic.

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 are the genotypes and relative advantages Acella™ Chemically Competent Cells and Acella™ pLysS Chemically Competent Cells?

Acella™ Chemically Competent Cells: F^- *ompT* $hsdS_B$ (r_B^- m_B^-) *gal dcm* (DE3) *ΔendA ΔrecA*
Acella™ pLysS Chemically Competent Cells: F^- *ompT* $hsdS_B$ (r_B^- m_B^-) *gal dcm* (DE3) *ΔendA ΔrecA* pLysS (Cam^R)

Genotype	Advantage
DE3	A lambda derivative bacteriophage that carries the gene for T7 RNA polymerase under the control of the *lacUV5* promoter (inducible by IPTG)
pLysS	A plasmid that expresses low levels of T7 lysozyme and is a natural inhibitor of T7 polymerase. It reduces basal levels of expression of recombinant genes and improves expression of toxic genes.
Cam^R	This denotes chloramphenicol resistance and is a selection marker of pLysS plasmid. We recommend a working concentration of 35 µg/ml for maintenance of pLysS plasmid.
ompT	Deficiency in the *ompT* protease, which results in higher yields of intact recombinant proteins
$hsdS_B$ (r_B^- m_B^-)	Allows cloning of DNA without cleavage by endogenous restriction endonucleases
ΔendA	Complete deletion of *endA* gene (DNA specific endonuclease I) shown to improve yield and quality of DNA from plasmid minipreps.
ΔrecA	Complete deletion of *recA* 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™ Chemically Competent Cells bacterial strain used?

Their high transformation efficiency and *ΔendA ΔrecA* 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™ Chemically Competent 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 plasmid DNA after only three hours of miniprep culture. All 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™ Chemically Competent Cells attain?

Edge BioSystems guarantees >2 x 10^8 colonies / µg pUC19, although transformation efficiencies are usually about 5 x 10^8 colonies / µg pUC19. As with any other competent cell, the transformation efficiency will decrease with larger plasmids or ligated DNA.

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

Most 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, solubility may be an issue, since some proteins are insoluble and aggregate in inclusion bodies when expressed in *E. coli* cells. 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™ Chemically Competent Cells for non-research purposes?

Edge BioSystems provides these materials for research purposes only. Acella™ Chemically Competent 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. Which Acella™ Chemically Competent strain best fits my needs?

The following chart can be used as a general guideline:

Chemically Competent Cell Strain	Description	Application
Acella™	(DE3) indicates that the host is a lysogen of λDE3 and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter.	Recombinant protein production non-toxic to *E. coli,* high quality plasmid preparation with short culture time (3 hours) for plasmid miniprep.
Acella™ pLysS	Coupled with the response above, the addition of pLysS indicates plasmid encoding small amounts of T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase and lowers the basal levels of recombinant protein before induction.	Recombinant protein production that may be toxic to *E. coli* or lower cell growth and viability. In the presence of detergents, small levels of lysozyme will help break the cell wall, which facilitate cell lysis once the protein is already expressed. Note that the pLysS plasmid may be present in plasmids preparations from this strain.

<u>Protein Expression</u>

1. Is it possible to directly clone, propagate and express into Acella™ Chemically Competent Cell strains?

Yes. Edge Biosystems' Acella™ Chemically Competent Cells strains have a transformation efficiency of >2 x 10^8, usually as high as 5 x 10^8, 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 lower transformation efficiency. The complete deletion of *endA* and *recA* genes makes this strain ideal for cloning purposes and DNA isolation. Since Acella™ Chemically Competent 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™ Chemically Competent Cells good for protein expression?

Yes, with the exception of the added *ΔendA ΔrecA* genotype, the Acella™ Chemically Competent Cells are identical to standard BL21 (DE3). BL21 cells naturally lack *ompT* and Lon proteases improving stability of synthesized proteins. BL21 (DE3) expresses T7 polymerase after induction with IPTG, therefore promoting the transcription of genes under the T7 promoter (for example, those in pET plasmids).

3. What proteins are the Acella™ Chemically Competent Cells strains good at expressing?

The expression properties of Acella™ Chemically Competent Cells strains 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.

4. How is expression controlled?

Acella™ Chemically Competent Cells strains are designed for high-level protein expression using T7 RNA polymerase-based expression systems. Acella™ pLysS Chemically Competent Cells strains 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 an Acella™ pLysS Chemically Competent Cell strain and a T7 *lac* promoter.

Ligation

1. Does ligated DNA need to be diluted before adding it to Acella™ Chemically Competent Cells?

No, ligated DNA does not require dilution prior to transformation.

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 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.

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™ Chemically Competent Cells use?

Acella™ Chemically Competent 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.

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Freezing and Thawing

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

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

Clone Yield Potential

1. Is it possible to predict the cumber 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™ Chemically Competent Cells have been thoroughly controlled for quality and are guaranteed to exceed transformation efficiencies of 2×10^8 colonies/μg 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 2×10^8 colonies/μg pUC19 supercoiled DNA. We test the presence of the pLysS plasmid in Acella™ pLysS Chemically Competent Cells by plating them in the presence of chloramphenicol.

Growth Conditions

1. How does the growth of Acella™ Chemically Competent Cell strains differ from other BL21 strains currently on the market?

Acella™ Chemically Competent 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™ Chemically Competent 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 after the heat shock step. 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:

Ingredients	Final Concentration
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 before the recovery?

1 ml SOC added directly to the 1.5ml tube of competent cells followed by incubation at 300 rpm at 37°C will lower the transformation efficiency.

5. Can I transform the 96-well plate using a thermocycler?

We have tested the transformation efficiency of our 96-Well plates using a thermocycler with a ramp of 1.5°C per second (with actual times of 28 seconds from 0°C to 42°C and 110 seconds from 42°C to 0°C) and the transformation efficiency was 40% of the standard efficiency. Using 2 thermocyclers and transferring the plate by hand, the transformation efficiency was 60% of the standard efficiency. In both cases, the cells were recovered in 0.5ml of SOC in a 2ml deep well plate at 300 rpm, 37°C.

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.

4. How do I calculate the 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)

5. How are cells made competent?

Cells can be made competent either 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.