



Frequently Asked Questions for ElectroEB10B Competent Cells

About Your ElectroEB10B Competent Cells

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 tube containing the product, there are other distinguishing labels on the packaging that make identification easy.

2. What is the genotype and relative advantage(s) of ElectroEB10B Competent Cells?

ElectroEB10B: F⁻ *mcrA* Δ(*mmr*-*hsdRMS*-*mcrBC*) Φ80*lacZ*Δ*M15* Δ*lacX74* *recA1* *endA1* *araD139* Δ(*ara*, *leu*)7697 *galU* *galK* λ⁻ *rpsL* *nupG* *tonA*.

Genotype	Advantage
<i>lacZ</i> Δ <i>M15</i>	Allows blue/white screening through β-galactosidase complementation.
<i>recA1</i>	Eliminates general DNA recombination.
<i>endA1</i>	Improves yield and quality of DNA in plasmid preparations.
<i>mcrA</i> , <i>mcrBC</i> , <i>mmr</i>	Allows cloning of methylated DNA including mammalian and plant genomic DNA.
<i>hsdRMS</i>	Construction of more representative genomic libraries.
<i>tonA</i>	Confers resistance to bacteriophages T1 and T5.

3. For what purposes are your ElectroEB10B Competent Cell bacterial strain used?

Faster growing than EB5Alpha, these cells are ideal for demanding cloning needs and for the generation of large plasmid-based libraries and cloning methylated DNA. Their high efficiency makes them the cells of choice for cloning experiments using limiting amounts of DNA, especially since it tolerates ligated genomic DNA. Blue/white color selection facilitates screening of positive clones. ElectroEB10B Competent Cells are able to transform larger sized inserts allowing the construction of BAC and P1 clones.

4. What efficiencies do your competent cells attain?

Edge BioSystems guarantees >10¹⁰ colonies / μg pUC19. As with any other competent cell, the transformation efficiency will decrease with extremely large plasmids or ligated DNA.

5. What are the advantages of using ElectroEB10B Competent Cells?

ElectroEB10B Competent Cells offer distinct advantages in particular applications. Blue/white screening and high transformation efficiencies make them ideal for all demanding cloning applications and generation of very large plasmid libraries. Their genetic background provide excellent plasmid yield and quality with peace of mind for phage T1 and T5 resistance. The high efficiency also allows the cloning of limiting amounts of DNA. ElectroEB10B Competent Cells also can transform larger clones and clones containing methylated DNA.



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6. Can I use my ElectroEB10B Competent Cells for non-research purposes?

This kit is intended for internal research use only by the purchaser. It is not to be used for human diagnostic purposes. In addition, “research use only” means that this kit and all of its contents are excluded from resale,

repackaging or use for the making or selling of any commercial product or service without the written approval from Edge BioSystems.

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, 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. Field strengths of greater than 15 kV/cm are usually recommended for bacteria.

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 which 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 ElectroEB10B?

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.

Protein Expression

1. Are ElectroEB10B Competent Cells good for protein expression?

ElectroEB10B Competent Cells are best suited for applications that include a need for increased plasmid yield and quality, ultra efficient blue/white screening, generation of very large plasmid libraries and generating clones from methylated DNA. Edge BioSystems proudly offers its Ultra BL21 (DE3) and Ultra BL21 (DE3) pLysS Competent Cells, which have proven to be very successful in protein expression applications.

Ligation

1. Does ligated DNA need to be diluted or desalted before adding it to ElectroEB10B Competent 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.



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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. Before electroporation, the DNA should be diluted or precipitated as high DNA concentration in the electroporation can cause arcing.

3. How does transformation efficiency of supercoiled pUC19 compare with cut/ligated DNA?

Supercoiled DNA will transform at a 10-fold higher efficiency than cut/ligated DNA.

Freezing and Thawing

1. Can I reuse my competent cells once they thaw?

Freeze/thaw cycles will lower transformation efficiencies. If you want to use a tube for several transformations, we recommend quickly aliquoting the number of samples required and refreezing the tube in a dry ice:ethanol bath. Every time a tube is taken from -80°C storage conditions, the transformation efficiency is affected. Therefore, for optimal efficiencies a tube should not be reused.

2. 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 6 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 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. ElectroEB10B Competent 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.

Quality Control

1. What quality control is performed to test the transformation efficiency of these 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.

Growth Conditions

1. How does the growth of the ElectroEB10B Competent Cells strain differ from other commonly used cells?

ElectroEB10B Competent Cells are a K12 derivative with similar growth. Generally, overnight incubation of plated transformed cells in LB medium and antibiotic at 37°C results in medium size colonies (about 1mm in diameter). ElectroEB10B Competent Cells will grow faster than EB5Alpha (and other DH5 α derivatives).



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Transformation and Recovery

1. What is the purpose of recovery?

After bacteria have been transformed with an antibiotic-resistant plasmid, recovery in medium lacking the antibiotic allows for synthesis of the antibiotic resistance prior to plating them in the presence of antibiotic. The importance of recovery depends on the antibiotic used. For example, recovery is essential for kanamycin-resistant plasmids; whereas, ampicillin-resistant plasmid recovery improves the transformation efficiency 2-5 fold.

2. How important is SOC medium for recovery when using these competent bacterial cells?

Glucose and magnesium when present in SOC seem to improve transformation efficiency. Our transformation efficiencies are calculated using a standard 60-minute incubation with SOC after the transformation. Use of LB medium for recovery will result in reduced transformation efficiencies.

3. 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

4. Can I recover my cells in less than 1 hour?

Yes, but anything less than 45 minutes can greatly reduce the number of colonies.

5. 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 usually refers to the number of cells that are transformed by 1 μ g DNA, usually a supercoiled plasmid.

4. How do I calculate the transformation efficiency?

$(\text{cfu on control plate}) / (\text{ng of supercoiled vector}) \times (10^3 \text{ ng} / \mu\text{g}) \times (\text{final dilution}) = \text{cfu} / \mu\text{g DNA}$

(Note: cfu = colony forming units)



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5. How are cells made competent?

Cells can be transformed either chemically or by electroporation. Chemical competency (ability to introduce DNA into cells) 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.