

## Frequently Asked Questions for EB5Alpha Competent Cells

### About Your EB5Alpha 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 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. What is the genotype and relative advantage(s) of EB5Alpha Competent Cells?

EB5Alpha:  $F^{\prime} \Phi 80 lacZ \Delta M15 \Delta(lacZYA-argF)U169 recA1 endA1 hsdR17(r_k^{-}, m_k^{+}) phoA supE44 thi-1 gyrA relA1 tonA$

Genotype	Advantage
<i>lacZ</i> $\Delta$ M15	Allows blue/white screening through $\beta$ -galactosidase complementation.
<i>recA1</i>	Eliminates general DNA recombination.
<i>endA1</i>	Improves yield and quality of DNA in plasmid preparations.
<i>hsdR17</i> ( $r_k^{-}, m_k^{+}$ )	Represents restriction negative and modification positive phenotype and allows cloning of DNA without cleavage by endogenous restriction endonucleases.
<i>relA1</i>	Permits RNA synthesis in the absence of protein synthesis.
<i>tonA</i>	Confers resistance to bacteriophages T1 and T5.

#### 2. For what purposes are your EB5Alpha Competent Cell bacterial strain used?

These cells are ideal for most cloning needs and for the generation of plasmid-based libraries. Their high efficiency makes them the cells of choice for cloning experiments using limiting amounts of DNA in either a high-throughput or single use format. Blue/white color selection facilitates screening of positive clones.

#### 4. What efficiencies do your competent cells attain?

Edge BioSystems guarantees  $>10^9$  colonies /  $\mu$ g pUC19. As with any other competent cell, the transformation efficiency will decrease with larger plasmids or ligated DNA.

#### 5. What are the advantages of using EB5Alpha Competent Cells?

EB5Alpha Competent Cells offers distinct advantages in particular applications. Blue/white screening and high transformation efficiencies make them ideal for all cloning applications and generation of libraries. They are especially



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suitable for high-throughput cloning applications. Their genetic background provide excellent plasmid yield and quality with peace of mind for phage T1 and T5 resistance.

### 5. Can I use my EB5Alpha 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.

### Protein Expression

#### 1. Are EB5Alpha Competent Cells good for protein expression?

EB5Alpha Competent Cells are best suited for applications that include a need for increased plasmid yield and quality, ultra efficient blue/white screening for cloning and generation of cDNA libraries. Although EB5Alpha cells can be used for protein expression, 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 EB5Alpha Competent Cells?

No, ligated DNA does not require dilution or desalting 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.

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

We compared transformation efficiencies of supercoiled pUC19 vs. ligated pUC19 after single and double cut. We also tested if heat activation of the ligase improved transformation efficiency. The results are shown below. Please note that in order to make the experiments comparable, transformations were not done according to our standard protocols. Transformation efficiencies with standard protocols exceed  $10^9$  cfu/ $\mu$ g pUC19.

Material (pUC19)	Transformation Efficiency	% Efficiency Relative to Supercoiled pUC19
Supercoiled pUC19	$5 \times 10^8$	100
Single cut pUC19, heat inactivated ligation	$6.2 \times 10^8$	100
Single cut pUC19	$5.4 \times 10^8$	100
Double cut pUC19, heat inactivated ligation	$5.4 \times 10^7$	10.8
Double cut pUC19	$3.9 \times 10^7$	7.8



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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 3 months, when properly stored at -70°C. Upon receipt of your cell shipment, cells should immediately be placed at -70°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. EB5Alpha Competent Cells have been thoroughly controlled for quality and are guaranteed to exceed transformation efficiencies of  $10^9$  colonies/ $\mu$ g pUC19 supercoiled DNA.

### Quality Control

#### 0. 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  $2 \times 10^9$  colonies/ $\mu$ g pUC19 supercoiled DNA.

### Growth Conditions

#### 0. How does the growth of the EB5Alpha Competent Cells strain differ from other commonly used cells?

EB5Alpha Competent Cells is a K12 derivative with similar growth. Generally, overnight incubation of plated transformed cells in LB/antibiotic at 37°C results in medium size colonies (about 1mm in diameter).



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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 present in SOC seem to improve transformation efficiency. Our transformation efficiencies are calculated using a standard 60-minute incubation with SOC after the transformation.

#### 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?

The following table shows recovery done with 1ml SOC in 14ml round bottom culture tubes. The percentages are relative to 60 minutes of recovery:

<u>Minutes</u>	<u>Transformation efficiency using pET41 plating in LB/Kan</u>	<u>Transformation efficiency using pUC19 plating in LB/amp</u>
60	100	100
45	76	87
30	19	60
15	2	63
0	0	59

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

Yes, you can add 250µl SOC directly to the 1.5ml transformation tube and shake it at 300 rpm for 1 hour without altering the transformation efficiency.



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### 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 $\mu$ 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)

#### 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 “arching” during the electrical shock. Both methods provide cells that can be frozen for storage.

