EB5Alpha Competent Cells

Product	Cat. No.	Transformations
EB5Alpha Competent Cells, Tubes	83254	12

Description

EB5Alpha Competent Cells are chemically competent and have been manufactured using a proprietary technology that renders the cells highly efficient for DNA uptake. To utilize the cells at their highest efficiency, a recommended transformation protocol is included with each kit.

EB5Alpha Competent Cells are available in single use tubes that provide a simple and reliable method for high-efficiency, single use transformation. All kits include a test plasmid for quality control purposes. Cells are pre-dispensed in 50µl aliquots (tubes).

Full processing time for 1 tube (including recovery) is about 1 hour 20 minutes to ensure the highest level of transformation. Edge BioSystems guarantees transformation efficiencies that exceed 10^9 cfu/µg pUC19.

Genotype: F$^-$Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-,m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A *rel*A1 *ton*A.

Kit Components 83254

EB5Alpha Competent Cells	12 tubes
pUC19 Supercoiled DNA, 100ng/ml	1 tube

Quality Control

Each lot has been tested to assure high transformation efficiency using 10pg pUC19 supercoiled DNA and the recommended protocol. Transformation efficiency will exceed >10^9 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.
3. A 42°C water bath.
4. 14ml round bottom culture tubes (1 tube per single use aliquot).
5. LB-agar selective plates or selective liquid medium.

Storage Conditions

EB5Alpha Competent 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 for Tubes

1. Place single use tubes on ice and wait approximately 5 minutes until they thaw.
2. Pipette the DNA to be transformed to the bottom of the tube and mix by pipetting 50µl of air to the bottom of the tube. Control transformation: Dilute pUC19 supercoiled DNA 1:10 with sterile H_2O, then add 1µl of the diluted pUC19 supercoiled DNA to one of the tubes. Discard diluted pUC19 supercoiled DNA after use.

   ***Note*: Do not mix by pipetting up and down since that will lower the transformation efficiency.**
3. Incubate the tubes in ice for 10 minutes.
4. Transfer the tubes to a 42°C water bath, incubate for 40 seconds and transfer the tubes back to ice.
5. Incubate the tubes for 2 minutes in ice.
6. Transfer the cells into a 14ml round bottom culture tubes filled with 1ml of pre-warmed SOC medium and then shake at 300 rpm at 37°C for 1 hour.
7. Plate on pre-warmed LB-agar selective plates or inoculate into selective liquid medium. For the control transformation with pUC19 supercoiled DNA, plate 10µl on LB-ampicillin agar plates and expect 100 colonies (>10^9 cfu/µg pUC19).