Lucigen®
Simplifying Genomics

BigEasy®-TSA™ Electrocompetent Cells

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

Email: techserv@lucigen.com
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Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

BigEasy TSA Electrocompetent Cells are shipped on dry ice in one container, along with supercoiled Control pKanR DNA at 10 pg/µL and Recovery Medium (Table 1).

BigEasy TSA Electrocompetent Cells require storage at –80 ºC.

Table 1: **BigEasy TSA Electrocompetent Cells**

	Catalog #	Reactions
BigEasy TSA Electrocompetent Cells in SOLO packaging (1 reaction per tube) (≥ 2 x 10^{10} cfu/□g pKanR DNA) (Blue cap)	60224-1	6 (6 x 25 µL)
	60224-2	12 (12 x 25 µL)
	60224-3	24 (24 x 25 □L)
Control pKanR DNA (10 pg/ µL) Store at **-20 °C** or **-80 °C**	----	1 x 20 µL
Recovery Medium Store at **-20 °C** or **-80 °C**	---- ---- 80026-1	12 (1 x 12 mL) 24 (2 x 12 mL) 96 (8 x 12 mL)

BigEasy TSA Electrocompetent Cells

BigEasy TSA Electrocompetent Cells are designed for use with Lucigen's BigEasy v2.0 Linear Cloning Kit, containing the pJAZZ® vector. Although the pJAZZ linear vector can be propagated in most laboratory strains of *E. coli*, only Lucigen's BigEasy TSA strain will provide high transformation efficiency and induction of copy number.

The BigEasy TSA strain is derived from Lucigen's *E. cloni*(TM) 10G strain. These cells give high yield and high quality plasmid DNA due to the *end*A1 and *rec*A1 mutations. They contain the *mcr* and *mrr* mutations, allowing methylated genomic DNA that has been isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. BigEasy TSA Cells are also resistant to infection by phage T1 (*ton*A mutation). The *rps*L mutation confers resistance to streptomycin.

Differences between BigEasy TSA and BigEasy pTel™ Electrocompetent Cells

The BigEasy TSA Cells have an ampicillin gene integrated into the chromosome and no exogenous plasmids (see Table below). The original BigEasy pTel Cells had an integrated chloramphenicol gene and an exogenous plasmid that encoded gentamycin resistance.

Because of the conflicts in antibiotic resistance, the BigEasy TSA Electrocompetent Cells can NOT be used with the original pJAZZ-KA vector, and the BigEasy pTel Cells can NOT be used with the vector pJAZZ-OC.

Cells	Antibiotic Resistance	Compatible Vector
BigEasy TSA	ampicillin^R	pJAZZ-OC (chloramphenicol^R) pJAZZ-OK (kanamycin^R)
BigEasy pTel	chloramphenicol^R	pJAZZ-KA

		(kanamycin^R + ampicillin^R)

BigEasy TSA Genotype:

F- *mcr*A □(*mrr-hsd*RMS-*mcr*BC) □80*dlacZ*□M15 □*lac*X74 *end*A1 *rec*A1*ara*D139 □(*ara*, *leu*)7697 *gal*U *gal*K *rps*L (Str^R) *nup*G □- *tonA bla (Amp^R) sopAB telN antA*

- BigEasy TSA Cells are provided with supercoiled pKanR DNA at a concentration of 10 pg/µL as a transformation control.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB or other media may result in lower transformation efficiencies.

Preparation for Transformation

BigEasy TSA Electrocompetent Cells are provided in 25 µL□aliquots (SOLOs), sufficient for one transformation reaction each. Transformation is carried out in a 0.1-cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting		Alternate Settings (~ 20-50% lower efficiencies)
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts		1.0 mm cuvette 25 µF 200 Ohms 1600 – 2000 Volts

Suggested Electroporation Systems: Bio-Rad Micro Pulser #165-2100; Bio-Rad E. coli Pulser #165-2102; Bio-Rad Gene Pulser II #165-2105; BTX ECM630 Electroporation System; Eppendorf Model 2510.

To ensure successful transformation results, the following precautions must be taken:

- **The pJAZZ® ligation reaction must be heat killed at 70 ºC for 15 minutes before transformation.**
- Electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results are obtained with cuvettes from BTX (Model 610), Eppendorf (Cat. #940001005), or BioRad (Cat. #165-2089). Users have reported much lower transformation efficiencies using Lucigen cells with Invitrogen cuvettes (Cat. # 65-0030).
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB or other media will result in lower transformation efficiencies.

Use **LB Lennox** agar plus antibiotic for plating cells ("LB Lennox+CXI" for pJAZZ-OC or "LB Lennox + Kan" plates for pJAZZ-OK. See Media Recipes). LB Lennox Agar is used to maximize colony size. Cells may be plated on LB Miller or other common media—colonies will be noticeably smaller upon comparison but adequate for the vast majority of intents and purposes.

- Optional transformation control reactions include electroporation with 1 µL (10 pg) of supercoiled pKanR DNA.

Transformation Protocol

1. Prepare Agar plates. LB Lenox Agar is used to maximize colony size. Cells may be plated on LB Miller or other common media—colonies will be noticeably smaller upon comparison but adequate for the vast majority of intents and purposes.

2. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use of SOC or other media.

3. Place electroporation cuvettes (0.1 cm gap) on ice.

4. Remove BigEasy TSA Electrocompetent Cells from the -80 °C freezer and thaw **completely** on wet ice (10-15 minutes).

5. Add 1 µL of the heat-denatured pJAZZ ligation reaction to the 25 µL of cells on ice. **Failure to heat-inactivate the ligation reaction will prevent transformation.** Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2 µL of ligation mix may cause electrical arcing during electroporation.

5. Carefully pipet 25 µL of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.

6. Within 10 seconds of the pulse, add 975 µL of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.

7. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.

8. Spread up to 100 µL of transformed cells on LB Lennox+ CXI or YT + Kan agar plates. For the pKanR control, use LB Lennox+kanamycin (30 µg/mL).

9. Incubate the plates overnight at 37 °C.

10. Pick white colonies at random and grow in TB medium containing 12.5 □g/mL chloramphenicol plus 1X Arabinose Induction Solution (if desired).

Media Recipes

Media Recipes

LB Lennox Agar Plates

Per liter:
10 g tryptone
5 g yeast extract
5 g NaCl
15 g agar

Medium for Growth of Transformants
LB Miller

Per liter: 10 g tryptone
5 g yeast extract
10 g NaCl

Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB

Per liter: 11.8 g tryptone
23.6 g yeast extract
9.4 g dipotassium hydrogen phosphate (anhydrous)
2.2 g potassium dihydrogen phosphate (anhydrous)
0.4% glycerol

Add all components to deionized water. Autoclave and cool to 55 °C.

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