BigEasy® TSA™
Electrocompetent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

Lucigen® Corporation
Advanced Products for Molecular Biology

MA042 v1.3

BigEasy® TSA™ Electrocompetent Cells

Contents

Ordering Information

Table 1: **BigEasy TSA Electrocompetent Cells**

	Catalog #	Reactions
BigEasy TSA Electrocompetent Cells in SOLO packaging (1 reaction per tube) ($\geq$ 4 x 10^{10} cfu/μg pKanR DNA)	60224-1	6 (6 x 25 μl)
	60224-2	12 (12 x 25 μl)
	60224-3	24 (24 x 25 μl)
Control pKanR DNA (1 ng/μl) Store at **-20°C** or **-80°C**	----	1 x 5 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)
YT Agar (powder)		

Components & Storage Conditions

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

IMPORTANT: BigEasy TSA Electrocompetent Cells require storage at –80° C.

BigEasy® TSA™ Electrocompetent Cells

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*® 10G strain. These cells give high yield and high quality plasmid DNA due to the *end*A1 and recA1 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 Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*dlacZ*ΔM15 Δ*lac*X74 *end*A1 *rec*A1*ara*D139 Δ(*ara*, *leu*)7697 *gal*U *gal*K *rps*L *nup*G λ- *tonA bla (Amp^R) sopAB telN antA*

- BigEasy TSA Cells are provided with supercoiled pKanR DNA at a concentration of 1 ng/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.

BigEasy® TSA™ Electrocompetent Cells

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		1.0 mm cuvette
10 µF		25 µF
600 Ohms		200 Ohms
1800 Volts		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 70ºC 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 **YT** agar plus antibiotic for plating cells ("YT+CXl" for pJAZZ-OC or "YT + Kan" plates for pJAZZ-OK. YT Agar powder is included with the cells. See Media Recipes).

Colonies do not grow well on LB agar plates.

Optional transformation control reactions include electroporation with 1 µl of a 1:100 dilution of the supplied supercoiled pKanR DNA (10 pg total).

Transformation Protocol

1. Prepare YT Agar from powder included with cells. Colonies do not grow well on LB plates.
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 YT+ CXI or YT + Kan agar plates. For the pKanR control, use YT+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 µg/ml chloramphenicol plus 1X Arabinose Induction Solution (if desired).

Media Recipes

TB Culture Medium for Growth of Transformants

Per liter: 11.8 g Bacto-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 except glycerol to deionized water. Autoclave and cool to 55°C. Immediately before use, add 8 ml of filter-sterilized 50% glycerol.

YT+CXI or YT+Kan Agar Plates

Add the YT Agar powder provided with the kit to 500 ml of deionized water. Autoclave and cool to 55°C. Add the appropriate filter-sterilized antibiotic to the cooled medium (e.g., YT Kan is 15 mg kanamycin per 500 ml for 30 μg/ml kanamycin). For YT+CXI plates, add 6.25 mg chloramphenicol to 500 ml (12.5 μg/ml), 1.5 ml 100mM IPTG, and 5 ml 2% X-gal.

Temperatures of >55°C may destroy the antibiotics. Do NOT add antibiotics to hot media! Pour approximately 20-25 ml per petri plate.

YT Agar is per liter: 8 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 15 g agar, plus antibiotic.

YT Agar is available to purchase separately as 5 packets with catalog number 60025-1.

Related Lucigen Products

- BigEasy™ v2.0 Linear Cloning Kits
- CloneSmart® Blunt Cloning Kits
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kits
- CloneDirect™ Rapid Ligation Kit
- GC Cloning Kits
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kits
- cSMART™ cDNA Cloning Kits
- *E. cloni*® EXPRESS Electrocompetent Cells
- OverExpress™ Electrocompetent Cells