Lucigen
Reagent Components

Endura™ High-Yield Electrocompetent Cells (RUO)

Manual

IMPORTANT!
-80 °C Storage Required
Immediately Upon Receipt

LGC Diagnostics & Genomics

Contents

Purpose of this document

This document introduces Endura High-Yield Electrocompetent Cells, kit contents and storage conditions as well as guidance on how to electroporate with your plasmid of choice.

Introduction to Endura High-Yield Electrocompetent Cells

This product is intended for Research Use Only (RUO). Not for use in diagnostic procedures.

Endura High-Yield Electrocompetent Cells provide superior plasmid yields among commercially available strains. **To maximize plasmid yield, you must use our 40X Plasmid Enhancer. We recommend an initial titration of 0.5X to 4X. Failure to use the Plasmid Enhancer with the Endura High Yield Electrocompetent cells will result in significantly reduced plasmid yields.** Achieving maximum yield depends on optimization of media, oxygenation, plasmid size, replication origin, insert composition, and fermentation system.

Disclosed Genotype: *endA1 ΔtonA(fhuA) recA13 ara-14 galK2 lacY1 proA2 rpsL20(Str^R) xyl-5 leu thiE*

Applications: High-yield, high-quality plasmid production

Kit contents and storage conditions

Lucigen Endura High-Yield Electrocompetent Cells are packed in LGC SOLO format for your convenience:

Electrocompetent cells:

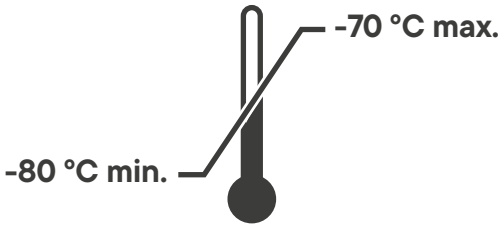
SOLO: 25 µL per vial, sufficient for one reaction

Minimum Transformation Efficiency:

Electrocompetent Cells: ≥ 1 x 10^{10} cfu/µg

The cells are shipped on dry ice in a single container, accompanied by Recovery Medium, 40X Plasmid Enhancer, and a supercoiled pUC19 DNA control at 10 pg/µL.

Competent cells require storage at –80 °C.



Product	Kit size	Catalogue number	Reagent description	Part number	Volume	Storage
Endura High-Yield Electrocompetent Cells (RUO)	12 transformations (SOLO)	60902-1	Endura High-Yield Electrocompetent Cells	F867917	(12 x 25 µL)	-80 °C
			*Recovery Medium	F98226-1	(1 x 12 mL)	-80 to +20 °C
			**Supercoiled pUC19 DNA (10 pg/µL)	F92078-1	(1 x 20 µL)	-80 to -20 °C
			40X Plasmid Enhancer	F848108-1	(1 x 12 mL)	-80 to -20 °C

Table 1. Components supplied in the Endura High-Yield Electrocompetent Cells kit, including details of volumes and storage conditions.

* LGC Lucigen Endura High-Yield Electrocompetent Cell kits contain 1 mL of Recovery Medium per transformation.
**Provided as a positive control for transformation. pUC19 contains ampicillin resistance.

Usage guidelines:

Preparation for electroporation

Electroporation is carried out in a 0.1 cm gap cuvette using 25 µL of Endura High-Yield Electrocompetent cells. Optimal settings for electroporation are listed in Table 2. Typical time constants are 3.5 to 4.5 msec. If your electroporator settings are not adjustable, simply use the E. coli or bacterial presets of your electroporator.

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	1400 – 1600 Volts

Table 2. Optimal settings for electroporation of Endura High-Yield Competent Cells.

Suggested electroporation systems:

Whilst any electroporation system should work with Endura High-Yield Electrocompetent Cells, Lucigen suggests the following 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 Electroporator 2510.

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

- Purified DNA should be dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in loss of the cells and DNA.
- Optional transformation control reactions may be performed with 1 µL (10 pg) of supercoiled pUC19 DNA included in kit.
- Thoroughly prechill microcentrifuge tubes and electroporation cuvettes on ice before use. Successful results can be obtained with cuvettes from many sources including BTX (Model 610), Eppendorf (Cat. #940001005), and BioRad (Cat. #165-2089).
- 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 SOC or other media will result in lower efficiencies.
- Prepare nutrient agar plus appropriate antibiotic for use at the end of the electroporation protocol. Low salt media, such as LB Lennox, is recommended.

Electroporation Protocol

1. Ensure Recovery Medium and 17 mm x 100 mm sterile culture tubes are readily available at room temperature. One tube is required for each planned transformation reaction.

2. Place electroporation cuvettes (0.1 cm gap) for each transformation reaction on wet ice.

3. Remove cells from the -80 °C freezer without removing the box from the freezer and place the tube on wet ice until the cells are completely thawed. This should take 10-20 minutes. Thaw one tube per transformation event.

4. Once thawed, mix cells by tapping gently. Add 1 µL of supercoiled DNA (10 pg-1ng).

   Initial cloning in the High Yield cells is not recommended. Other strains, including E. cloni 10G competent cells and Endura Electrocompetent Cells, are better suited for initial cloning experiments.

5. Carefully pipette the cells and supercoiled DNA mixture into a pre-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 as per recommendations in table 2 or use the bacterial presets of your electroporator.

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

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

8. Spread up to 100 µL of transformed cells on nutrient agar plates containing the appropriate antibiotic. To get single colonies from an electroporation of 1 ng of supercoiled DNA, the following plating regimen is recommended; plate 10 µL of a 1:100 dilution, 10 µL of a 1:10 dilution, or 1 µL outgrowth, 10 µL outgrowth, 50 µL outgrowth and 100 µL outgrowth. The greater the amount of DNA, the more potential need for dilution of the outgrowth.

9. Incubate the plates overnight at 37 °C. If cell density is too high, cells may be sub-cultured on a fresh antibiotic plate to get individual colonies.

Bacterial Cell Growth Recommendations

Transformed clones can be further grown in any rich culture medium with appropriate selective antibiotic, for example Luria-Bertani (LB) and Terrific Broth. When grown in other media, take care to consider proA2, leu, and thiE genotypic features of the strain. **To maximize plasmid yield, you must use our 40X Plasmid Enhancer solution. We recommend an initial titration of 0.5X to 4X. If yield continues to rise at 4X you can continue to increase the concentration of Plasmid Enhancer solution. Failure to use the Plasmid Enhancer solution with the Endura High-Yield Electrocompetent Cells will result in significantly reduced plasmid yields.** Maximum yield will require optimisation of media, amount of induction solution and thorough oxygenation (250-275 rpm in culture tubes or small flasks) and is dependent on plasmid size, origin of replication and insert as well as fermentation system.

Example Protocol

1. Remove plates from incubator and place on bench*. Plates may be stored at 4 °C; however, fresh transformations will yield better results (See Capaldo-Kimball F. and Barbour S.D. 1971, Capaldo-Kimball F., et al. 1974).

2. Obtain 250 mL autoclaved Erlenmeyer flasks.

3. Add 10 mL LB to flasks.

4. Add antibiotic corresponding to selection marker on plasmid. Typically antibiotics are taken from 1000x stocks and used at 1X. See Green and Sambrook, 2012 for recommended antibiotic concentrations.

5. Add 250 µL 40X Plasmid Enhancer (F848101) to the 10 mL LB plus antibiotic so that final concentration is 1X.

6. Pick one colony from each unique transformation plate with a sterile inoculation loop and mix in corresponding flask*.

7. Grow culture 16 to 18 hours at 37 °C with shaking at 250 rpm.

8. Extract plasmid DNA using commercially available purification kits following manufacturer's instructions. Zymo Research (https://www.zymoresearch.com/pages/bacterial-plasmid) and Qiagen (https://www.qiagen.com/us/product-categories/discovery-and-translational-research/dna-rna-purification/dna-purification/plasmid-dna) offer multiple plasmid purification kits. **Be sure to be aware of the manufacturer's specifications regarding capacity as the Endura High-Yield Electrocompetent Cells may exceed capacity expectations.**

*Alternatively, a glycerol stock may be made for each unique transformation and cultures may be started from a glycerol cell bank. See Green and Sambrook, 2012 and Q5D guidelines for procedural recommendations on cell banking.

References

Green, M.R. and Sambrook, J. 2012. *Molecular cloning: A laboratory manual*. 4th ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

International Conference on Harmonisation (1997) *Derivation and characterisation of cell substrates used for production of biotechnological/biological products Q5D.* Geneva: ICH Secretariat. Available at: https://database.ich.org/sites/default/files/Q5D%20Guideline.pdf

Capaldo-Kimball F. and Barbour S.D. 1971. *Involvement of Recombination Genes in Growth and Viability of Escherichia coli K-12*. Journal of Bacteriology: 106 (1), 204-212.

Capaldo-Kimball F., Ramsey G. and Barbour S.D. 1974. *Analysis of the Growth of Recombination-Deficient Strains of Escherichia coli K-12*. Journal of Bacteriology: 118 (1), 242-249.

Technical Support

LGC 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, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual 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.

LGC Technical Support email: techsupport@lgcgroup.com

Media recipes

LB Lennox agar plates

Per litre:

10 g tryptone

5 g yeast extract

5 g NaCl

15 g agar

Medium for growth of transformants

LB Miller

Per litre:

10 g tryptone

5 g yeast extract

5 g NaCl

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

TB

Per litre:

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 deionised water. Autoclave and cool to 55 °C.

LB Lennox agar is used to maximise colony size. Cells may be plated on LB or other common media — colonies will be noticeably smaller upon comparison but adequate for the vast majority of intents and purposes.

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