Manual

HI-Control BL21(DE3) and HI-Control 10G Chemically Competent Cells

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-80 ºC storage required
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

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1. Product description

1.1 HI-Control 10G Chemically Competent Cells

HI-Control™ 10G cells are an *E. coli* strain optimised for high efficiency transformation. They are ideal for cloning and propagation of plasmid clones. They give high yield and high quality plasmid DNA due to the *end*A1 mutation. HI-Control 10G cells harbor a single-copy BAC plasmid carrying an engineered *lacI*^{q1} repressor allele. The *lacI*^{q1} allele expresses approximately 170-fold more *lac* repressor than does the wild-type *lacI* gene[1]. The HI-Control 10G strain does not contain T7 RNA polymerase, and the excess *lac* repressor in this strain further minimises any background transcription by the bacterial polymerase.

1.2 HI-Control BL21(DE3) Chemically Competent Cells

The HI-Control BL21(DE3) cells are a derivative of BL21(DE3) with improved control over target gene expression. BL21(DE3) is the T7 host strain most commonly used for expression of cloned genes from the bacteriophage T7 promoter. This strain is a lysogen of λDE3, which harbors the T7 bacteriophage RNA polymerase gene under the control of the inducible *lacUV5* promoter. The *lacUV5* promoter is a variant of the *lac* promoter that is inducible to higher levels than its wild-type counterpart, but it also suffers from a higher basal level of activity. This basal expression of T7 RNA polymerase can lead to undesired expression of target genes cloned under a T7 promoter prior to induction. Such 'leaky' expression can lead to difficulty maintaining clones in the expression host, particularly if the target gene encodes a deleterious protein. To counter this, some common T7 expression vectors harbor a copy of the *lacI* gene, encoding the *lac* repressor protein[2, 3]. The *lac* repressor protein is responsible for maintaining inducible control over the *lacUV5* promoter as well as the T7-*lac* promoter on the vector. This increased copy number of *lacI* provides only partial protection against leaky expression.

The HI-Control BL21(DE3) cells contain a single-copy BAC plasmid harboring a specially engineered version of the *lacI*^{q1} repressor allele. The *lacI*^{q1} allele expresses approximately 170-fold more *lac* repressor protein than the wild-type *lacI* gene[1], or about 10-fold more repressor than expected when *lacI* is harbored on the expression vector. The increased pool of *lac* repressor in HI-Control BL21(DE3) cells maintains tight control over the expression of T7 RNA polymerase from the *lacUV5* promoter, reducing leaky expression of genes cloned under a T7 promoter. The excess repressor in this strain is also sufficient to bind to the *lac* operator on the T7 vectors, providing an additional level of control over expression from the T7 promoter. The abundant *lac* repressor does not interfere with the induction of T7 RNA polymerase or target gene expression by IPTG.

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2. Product specifications

Specification	HI-Control 10G	HI-Control BL21(DE3)
Transformation efficiency:	HI-Control 10G Chemically Competent Cells produce ≥ 1 x 10^9 cfu/µg supercoiled pUC19 DNA.	HI-Control BL21(DE3) Chemically Competent Cells produce ≥ 1 x 10^7 cfu/µg supercoiled pUC19 DNA.
Genotype	*mcrA* Δ(*mrr-hsdRMS-mcrBC*) *endA1 recA1* φ80d*lacZ*ΔM15 Δ*lac*X74 *araD139* Δ(*ara,leu*)7697 *galU galK rpsL* (Str^R) *nupG* λ^- *tonA* Mini-F $lacI^{q1}$ ($Gent^R$)	F^- *ompT hsdSB* (*rB^- mB^-*) *gal dcm* (DE3) Mini-F $lacI^{q1}$ ($Gent^R$)

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
HI-Control 10G Chemically Competent Cells (white cap)	12 reactions (SOLOs*)	60110-1	HI-Control 10G Chemically Competent Cells	F96703	12 (12 x 40 µL)
			pUC 19Transformation Control**	F92078-1	20 µL
			Recovery Media***	F88912-1	12 (1 x 12 mL)
HI-Control BL21(DE3) Chemically Competent Cells (gray cap)	12 reactions (SOLOs*)	60435-1	HI-Control BL21(DE3) Chemically Competent Cells	F96704	12 (12 x 40 µL)
			pUC 19Transformation Control**	F92078-1	20 µL
			Recovery Media***	F98226-1	12 (1 x 12 mL)

* SOLOs are packaged as one transformation per tube

** Supercoiled pUC19 DNA (10 pg/µL) should be stored at -20 to -80 °C.

*** Recovery Media should be stored at -20 to -80 °C. It is also available separately as Catalogue #80026-1 (96 mL; 8 x 12 mL)..

4. Storage conditions

All HI-Control Competent Cells require storage at -80 °C.

5. Heat shock transformation of HI-Control Chemically Competent Cells

HI-Control Chemically Competent Cells are provided in 40 µL aliquots, sufficient for a single transformation. Transformation is performed by incubation on ice followed by heat shock at 42 °C. For maximal transformation efficiency, the heat shock is performed in 15 mL polypropylene culture tubes (17 x 100 mm). The use of other types of tubes may dramatically reduce the transformation efficiency. To ensure successful transformation results, the following precautions must be taken:

- All culture tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.

5.1 Transformation protocol

1. Remove Recovery Medium from the freezer and bring to room temperature.
2. Remove HI-Control cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
3. Briefly centrifuge thawed plasmid DNA to collect the solution in the bottom of the tube.
4. Transfer the cells to a pre-chilled disposable polypropylene 15 mL culture tube (17 x 100 mm).
5. Add 1 µL of DNA (10 pg to 100 ng) to the cells. Stir briefly with pipette tip. Do **not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
6. Incubate culture tube containing cells and DNA on ice for 30 minutes.
7. Heat shock cells by placing the tube in a 42 °C water bath for 45 seconds.
8. Return the tube of cells to ice for 2 minutes.
9. Immediately following the 2 minute ice incubation, add 960 µL of room temperature Recovery Medium to the cells in the culture tube.
10. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
11. Plate 50 µL of transformed cells on LB agar plates containing the appropriate antibiotic. For pUC19 control transformations, HI-Control BL21(DE3) cells should yield >5 colonies, and HI-Control 10G cells should yield >500 colonies.
12. Incubate the plates overnight at 37 °C.

Transformed clones can be grown in LB, TB or any other rich culture. Growth in TB medium typically gives the highest culture density and yield of plasmid or protein. Glucose may be added to 0.5% final concentration to further minimise expression of the recombinant plasmid.

5.2 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 deionised 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 deionised water. Autoclave and cool to 55 °C.

6. References

1. Glascock CB and Weickert MJ, 1998, Using chromosomal *lacI*Q1 to control expression of genes on high-copy-number plasmids in *Escherichia coli. Gene* 223, 221.
2. Studier FW, Rosenberg AH, Dunn JJ and Dubendorff JW, 1990, Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* 185, 60.
3. Dubendorff JW and Studier FW, 1991, Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor. *J. Mol. Biol.* 219, 45.

7. Technical support and product guarantee

LGC, Biosearch Technologies™ 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.

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: Biosearch Technologies guarantees that this product will perform as specified for one year from the date of shipment.

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