



Autolytic *E. coli* Cell Lines

Instruction Manual

Catalog Numbers

NTC-DH5-AL_{HEAT}

NTC-DH5-AL_{IPTG}

NTC-BL21-AL_{HEAT}

NTC-BL21-AL_{IPTG}

Version 1

May 2009

Nature Technology Corporation.

4701 Innovation Drive Lincoln Nebraska, 68521

Telephone: (402) 323-6289

Fax: (402) 323-6292

Email: support@natx.com

Website: www.natx.com

General Information

Contents: 0.2 mL of glycerol stock shipped on dry ice.

Storage: Glycerol stocks should be stored at -80°C

Autolytic cell lines

Introduction

Nature Technology Corporation (NTC) has developed autolytic *Escherichia coli* host strains that express a chromosomal, integrated bacteriophage endolysin gene (λ R), encoding a peptidoglycan hydrolase (lysozyme) enzyme to permeabilize the bacterial cell wall. Expression of the endolysin is induced during growth by either a heat inducible (AL_{HEAT}) or IPTG inducible (AL_{IPTG}) promoter. The endolysin remains in the cytoplasm, where it is separated from its peptidoglycan substrate in the cell wall; hence the cells remain alive and intact and can be harvested by the usual methods (Carnes et al., 2009). Cell lysis can then be performed using a freeze thaw cycle or with Triton X-100/EDTA lysis solutions (Crabtree and Cronin, 1984; Studier, 1991).

NTC's autolytic cell lines are derivatives of the popular DH5 α and BL21 cells lines (Carnes et al., 2009). These autolytic cell lines are available in two versions that are compatible for use with a variety of plasmids or inducible protein expression vectors.

AL_{IPTG} cell lines contain chromosomally integrated tac promoter-expressed phage λ R endolysin regulated by the lac repressor. The AL_{IPTG} cell lines are IPTG inducible, and also compatible with lactose-based auto-induction strategies (Studier, 2005).

AL_{HEAT} cell lines contain chromosomally integrated pR-expressed phage λ R endolysin regulated by the temperature sensitive C1867 phage λ repressor. Heat inducible endolysin is tightly repressed at 30°C, and induced at 42°C (Carnes et al. 2009).

These cell lines can be used for autolytic purification of plasmid or protein. No reduction of growth rate, viability, plasmid yield or integrity, compared to production with *E. coli* DH5 α , was observed with the NTC3012 autolytic host strain using a 30-42°C inducible fermentation process (Williams et al., 2009). NTC3012 cells can be used in an acidic autolytic plasmid DNA extraction procedure to selectively extract plasmid DNA without releasing bulk genomic DNA and cell debris (Carnes et al., 2009)

In summary, NTC's autolytic *E. coli* host strains offer the following advantages:

- Simple regulated *E. coli* autolysis using chromosomally integrated stable cell lines
- Heat or IPTG-inducible phage λ R endolysin
 - Heat inducible λ R (tightly repressed at 30°C, induced at 42°C)
 - IPTG inducible λ R (tightly repressed in glucose media, induced with IPTG)
- Tight regulation prior to induction with integrated lac repressor (IPTG-inducible) or λ repressor (heat inducible)
- High-level Endolysin production post induction using optimized *zwf*- λ R cistron
- Derivatives of established strains for plasmid (DH5 α) or protein (BL21) production
- Streamlines and simplifies plasmid or protein extraction protocols (Carnes et al., 2009b)
- Compatible with a variety of plasmid selection markers including ampR, kanR, chlorR

Autolytic cell lines

Strain	Genotype	Induction	Catalog Number	Price
NTC3012	DH5 α att _{HK022} ::P _R -zwf- λ R, strR	37-42°C	NTC-DH5-AL _{HEAT} (Glycerol stock)	\$90.00
NTC3012 competent cells	DH5 α att _{HK022} ::P _R -zwf- λ R, strR	37-42°C	NTC-DH5-AL _{HEAT} -CC1 (10x100μL competent cells)	\$180.00
NTC40116	DH5 α att _{HK022} ::P _{tac} -zwf- λ R, strR	IPTG	NTC-DH5-AL _{IPTG} (Glycerol stock)	\$90.00
NTC40116 competent cells	DH5 α att _{HK022} ::P _{tac} -zwf- λ R, strR	IPTG	NTC-DH5-AL _{IPTG} -CC1 (10x100μL competent cells)	\$180.00
NTC4828	BL21 att _{HK022} ::P _R -zwf- λ R, strR	37-42°C	NTC-BL21-AL _{HEAT} (Glycerol stock)	\$90.00
NTC4828 competent cells	BL21 att _{HK022} ::P _R -zwf- λ R, strR	37-42°C	NTC-BL21-AL _{HEAT} -CC1 (10x100μL competent cells)	\$180.00
NTC40117	DH5 α att _{HK022} ::P _{tac} -zwf- λ R, strR	IPTG	NTC- BL21-AL _{IPTG} (Glycerol stock)	\$90.00
NTC40117 competent cells	DH5 α att _{HK022} ::P _{tac} -zwf- λ R, strR	IPTG	NTC- BL21-AL _{IPTG} -CC1 (10x100μL competent cells)	\$180.00

DH5 α : F- Φ 80lacZ Δ M15 Δ (lacZYA -argF) U169 *recA1 endA1 hsdR17*(τ_K^- , m_K^+) *gal phoA supE44* λ - *thi-1 gyrA96 relA1*

BL21: F- *ompT hsdSB* (*r_B*-*m_B*-) *gal dcm*

Bacterial propagation

The λ R integration in the autolytic cell lines is marked by streptomycin/spectinomycin (Strep/Spec) resistance (strR). This marker can be selected on standard *Escherichia coli* media such as LB supplemented with spectinomycin/streptomycin (35 μ g/mL each). The integrations are stable, and strR selection is not necessary.

Transformation of competent cells

- 1) Thaw competent cells on ice
- 2) Add plasmid DNA (1-3 μ L) and mix by gently flicking the tube with your fingers.
- 3) Incubate 30 minutes on ice
- 4) Add 0.5 mL SOC (**room temperature**) to the transformation tube
- 5) Shake 1-2 hours at 30°C
- 6) Transfer 20-100 μ L to an agarose plate (**prewarmed to room temperature**) containing the appropriate antibiotic (for selection of plasmid-borne selectable marker such as ampR, kanR, chlorR) and spread cells
- 7) Incubate plate at 30°C overnight
- 8) Isolate colonies

Autolytic cell line induction

- 1) Grow cells containing the plasmid of interest under non-inducing conditions

Growth of AL_{HEAT} cell lines must be performed at 30°C to prevent inactivation of the temperature sensitive λ repressor. This repressor is partially functional at 37°C and non functional at 42°C, allowing endolysin expression at these temperatures.

Growth of AL_{IPTG} cell lines prior to induction must be performed in the absence of IPTG to prevent endolysin induction. Many lots of tryptone used to make standard LB media contain detectable amounts of lactose which can induce the tac promoter. Inclusion of glucose to 0.2-1.0% in plates and media will prevent such induction (Studier, 2005).

- 2) Induce plasmid-encoded protein production if desired. This involves adding inducer for the expression vector if a plasmid-encoded protein is the desired product. For temperature or IPTG induced vectors, this can be coordinate with endolysin induction (using AL_{HEAT} or AL_{IPTG} cell lines, respectively).
- 3) Induce chromosome encoded endolysin production. If a pUC origin plasmid is the intended product, coordinately induce plasmid copy number and endolysin using the AL_{HEAT} cell lines by temperature shift to 42°C for >2 hrs.
- 4) Harvest cells. Cells may be stored at -80C and lysed using a freeze thaw cycle (Crabtree and Cronin, 1984; Studier, 1991). Cell lysis can also be performed on fresh or frozen cells with Triton X-100/EDTA lysis solutions (*e.g.*, STET lysis buffer; **Fig. 1**). Soluble cellular content may also be selectively released using acidic extraction (Carnes et al., 2009).

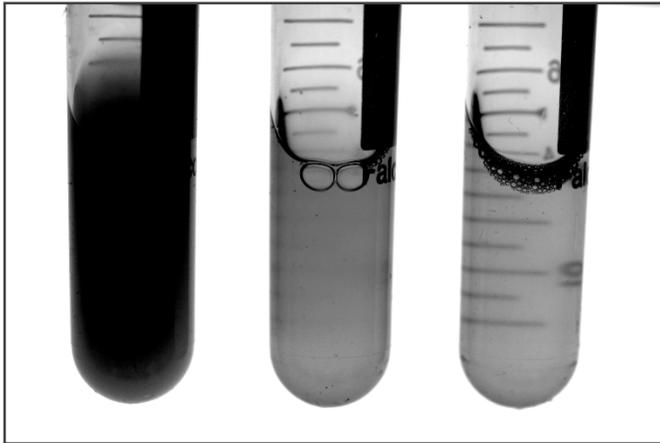


Figure 1: Endolysin can substitute for lysozyme in a STET/lysozyme process.

E. coli autolysis at 30 OD₆₀₀ in STET buffer (50 mM Tris, 50-100 mM EDTA, 8% sucrose, 2% Triton X-100, pH 8.0) after 30 minutes at room temperature. *Left*: STET suspension of non-autolytic DH5 α . *Middle*: STET suspension of non-autolytic DH5 α to which 2500 units Ready-LyseTM Lysozyme (Epicentre, Madison WI) was added. *Right*: STET suspension of autolytic DH5 α att_{HK022}::P_R-zwf- λ R, which lysed efficiently without addition of lysozyme.

SOC media (1 L)

4 g Glycerol
12 g Tryptone
24 g Yeast extract

Stir to dissolve

Add 10 mL of 250 mM KCl
Adjust pH to 7.0 with NaOH

Autoclave 20 min at 15 psi

Mix by swirling and once cooled to <60 °C add following:

10 mL of sterile (filtered or autoclaved) 1 M MgCl₂
20 mL of sterile (filtered or autoclaved) 20% (w/v) glucose

LB media (1 L)

10 g NaCl
10 g Tryptone
5 g Yeast extract
Stir to dissolve
pH to 7.0 with NaOH (optional)

Autoclave 20 min at 15 psi

Optional: Add 10 mL of sterile (filter or autoclaved) 20% (w/v) glucose (0.2% final concentration) to suppress lactose induction of tac promoter (some Tryptone lots contain trace lactose; Studier, 2005) during early growth (glucose will be depleted from media). To fully suppress lactose induction, add glucose to 1% final concentration.

LB agar (1 L)

10 g NaCl
10 g Tryptone
5 g Yeast extract
Stir to dissolve
pH to 7.0 with NaOH (optional)
Add 15 g Agar (not agarose)

Autoclave 20 min at 15 psi

Mix by swirling and add antibiotic as necessary once cooled to <60 °C

Optional: Add 10 mL of sterile (filtered or autoclaved) 20% (w/v) glucose to media prior to pouring plates.

References

Carnes AE, Hodgson CP, Luke J, Vincent J, Williams, J.A. (2009a) Plasmid DNA production combining antibiotic-free selection, inducible high yield fermentation, and novel autolytic purification. *Biotechnol Bioeng* Epub

Carnes AE, Hodgson CP, Williams JA. (2009b) Improved *E. coli* plasmid production strains. World Patent application WO2009025690

Crabtree S, Cronan JE. (1984) Facile and gentle method for quantitative lysis of *Escherichia coli* and *Salmonella typhimurium*. *J Bacteriol.* 158: 354-356

Studier FW. (1991) Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system. *J Mol. Biol.* 219: 37-44

Studier FW. (2005) Protein production by auto-induction in high-density shaking cultures. *Protein Expr Purif* 41:207-234

Williams JA, Luke J, Langtry S, Anderson S, Hodgson CP, and Carnes AE. (2009) Generic plasmid DNA production platform incorporating low metabolic burden seed-stock and fed-batch fermentation processes. *Biotechnol Bioeng* Epub

Patent and Licensing information

Limited License

Nature Technology Corporation (NTC) grants the end user (purchaser) of the autolytic cell lines NTC3012, NTC4828, NTC40116, and/or NTC40117 a nontransferable, non-exclusive license to use the plasmids for non-commercial research purposes only. These cell lines are intended for research use only by the purchaser. The cell lines cannot be resold, repackaged, or used for the making or selling of any commercial product or service without the written approval of Nature Technology Corporation.

Separate licenses are available from NTC for the express purpose of non-research use or applications of the NTC3012, NTC4828, NTC40116, and/or NTC40117 autolytic cell lines.

Product Use Limitations

The NTC3012, NTC4828, NTC40116, and/or NTC40117 autolytic cell lines are sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use.

Patent Information

NTC makes no representations that the use of the NTC3012, NTC4828, NTC40116, and/or NTC40117 autolytic cell lines will not infringe any patent, copyright, trademark, or other proprietary rights.

For more information, please contact:

Clague Hodgson

Nature Technology Corporation.

4701 Innovation Drive Lincoln Nebraska, 68521

Telephone: (402) 472-6530

Fax: 402-472-6532.

Email: support@natx.com