INSTRUCTIONS

XJ Autolysis™ *E. coli* strains

Catalog No. T5021, T5031, T5041, T5051, T3021, T3031, T3041, and T3051.

Highlights

- Simple and controlled autolysis of *E. coli*
- Strains can by lysed in minutes after harvesting
- Method compatible with most buffer systems
- Ideal for protein expression and purification, also applicable for extraction of nucleic acids
- Scale up to lyse more samples without increase in time or labor

Table of Contents

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GENERAL INFORMATION

Specifications

- **Protocol time**: 10 minutes
- **Lysis method**: enzymatic, by intracellularly expressed λ-endolysin
- **Efficiency**: 80-90% cells are lysed after a single freeze-thaw treatment
- **Convenience**: compatible with most buffer systems and with any other physical methods of lysis

Contents & Ordering

Products	Description	Format and Size	Catalog number
XJa Autolysis™	*E. coli* JM109 with chromosomally inserted λ lysozyme gene inducible by arabinose. Z-competent are chemically competent cells with high transformation efficiency.	**1 glycerol stock 1 ml 500x Arabinose**	**T5021**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3021**
XJa(DE3) Autolysis™	JM109(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysogen encodes chromosomally-encoded T7 polymerase and is therefore a suitable host for expression of recombinant proteins under the control of the T7 promoter, such as in the pET system.	**1 glycerol stock 1 ml 500x Arabinose**	**T5031**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3031**
XJb Autolysis™	*E. coli* BL21 with chromosomally inserted λ lysozyme gene inducible by arabinose.	**1 glycerol stock 1 ml 500x Arabinose**	**T5041**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3041**
XJb(DE3) Autolysis™	BL21(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysogen encodes chromosomally-encoded T7 polymerase and is therefore a suitable host for expression of recombinant proteins under the control of the T7 promoter, such as in the pET system.	**1 glycerol stock 1 ml 500x Arabinose**	**T5051**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3051**

Reordering

Products	Description	Format and Size	Catalog number
500x Arabinose	500x concentrated arabinose inducer. Contains $MgCl_2$ to improve growth of the autolysing strains. Sterile, ready to use. Storage: 25°C to -20°C (Contents: 1.5 M L-arabinose, 0.5 M magnesium chloride)	**1 X 1 ml**	**A2001-1**
		10 X 1 ml	**A2001-10**

Storage

Glycerol stocks are shipped at room temperature and should be frozen at -70°C to -80°C upon arrival. Do not place in liquid nitrogen. After freezing, the glycerol stock is stable for several years if never allowed to thaw. Multiple freezing and thawing will decrease viability of the glycerol stock. To withdraw cells from the vial, just remove a little of the material from the top of the frozen culture and return the tube back in the freezer promptly. Restreak the cells on LB agar plates and incubate overnight.
Z-competent cells are shipped frozen on dry ice and should be placed at -70°C to -80°C upon arrival. Do not place in liquid nitrogen. Thaw on ice immediately before use.

GENERAL DESCRIPTION

While there are many cell lysis methods available to scientists, unfortunately none of these methods combine all of the ideal features for simple, efficient, economical, and gentle lysis of *E. coli* cells. The *E. coli* XJ autolysing strains from Zymo Research were engineered to address this problem. Mild expression of a chromosomally encoded bacteriophage lambda R gene, encoding the lambda lysozyme, also known as lambda endolysin, is induced during growth. Cells are harvested intact while the peptidog lycan layer of the cell walls has been protected from digestion by the cytoplasmic membrane. The membrane is, however, amenable to disruption by a brief physico-chemical stress such as a freeze-thaw cycle after harvesting the cells (Figure1). The XJ Autolysis™ method is highly efficient and takes only minutes (unlike traditional multiple freeze-thaw cycles). It can be applied to any number of samples without increase in processing time and labor (unlike sonication or French-press), is reliable and repeatable (unlike lysozyme treatment), and finally, is fully compatible with a wide range of buffers. Additionally, it does not require use of any potentially interfering components such as detergents, commonly found in various lytic buffers.

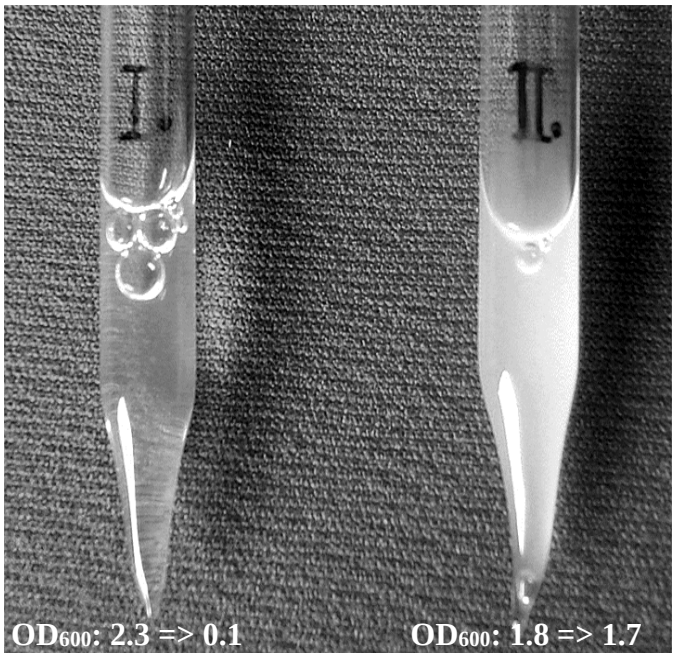
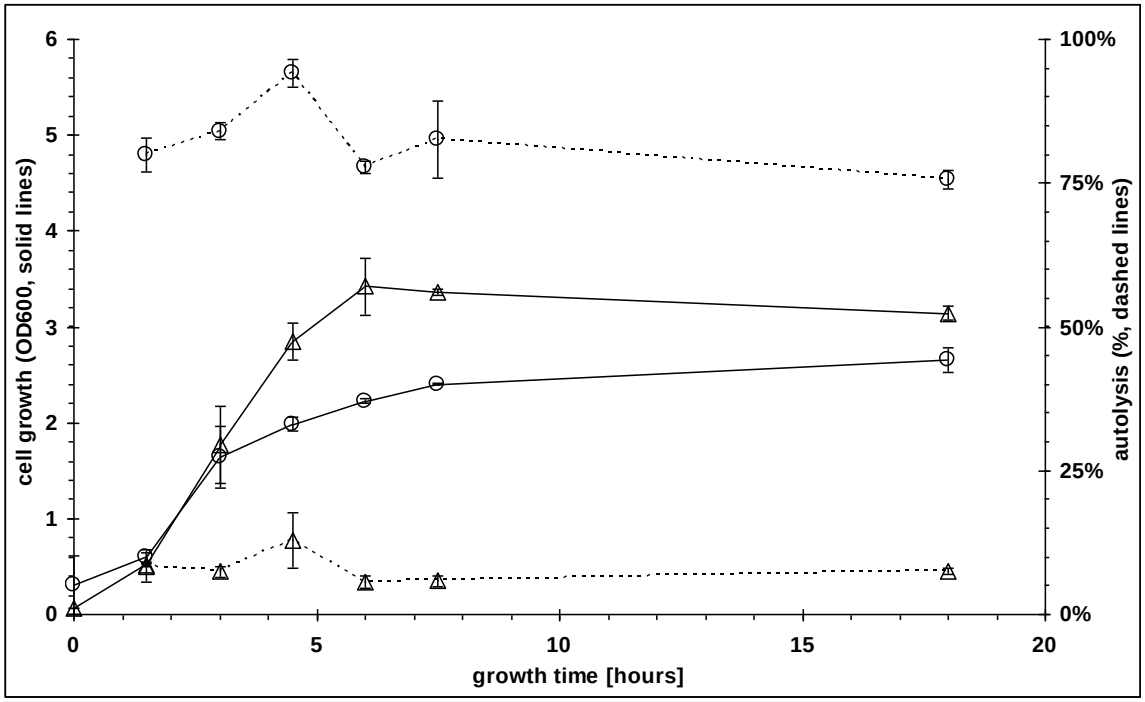


Figure 1. Autolysis of XJa cells. *E. coli* XJa cells (I) and control *E. coli* JM109 cells (II) were grown in LB for 24 hours. Autolysis was induced by arabinose addition. 4 mg of wet cells were resuspended in 1 ml of water, frozen on dry ice, and then incubated for 5 minutes on a 15°C water bath. The OD_{600} values indicate cell density changes before and after the freeze-thaw cycle. Similar results are obtained with the XJb strains (see www.zymoresearch.com for more experimental data).



Autolysis of *E. coli* XJa strain grown in LB media with 3 mM arabinose added at beginning of incubation. The chart shows the growth (open circle, solid line) and extent of autolysis (open circle, dashed line) of the autolysing strain XJa. For comparison, the growth (open triangle, solid line) and autolysis (open triangle, dashed line) of a control strain, *E. coli* JM109, is shown. The autolysing activity is defined as the amount of cell protein released after one freeze-thaw cycle, compared to the total protein in that sample. The total protein was measured after cell disruption by sonication at conditions which insured complete cell lysis. Similar results are obtained with the XJb strains (see www.zymoresearch.com for more experimental data).

PROTOCOLS

A: Transformation of Z-competent cells

The Z-Competent *E. coli* cells are pre-made chemically competent cells for simple and highly efficient *E. coli* transformation. Z-Competent *E. coli* cells are made chemically competent by a novel method which completely eliminates the need for heat shock and other downstream procedures. For transformation, simply mix DNA with Z-Competent cells, incubate on ice for 10-20 minutes, and spread on plates. The pre-made Z-competent cells are highly efficient (>10^8 transformants/µg pUC19) and can be used for cloning, sub-cloning, PCR fragment cloning, library construction, and other common molecular biology procedures. Pre-made Z-competent cells are supplied as a pack of 10 convenient 100 µl/tube single use aliquots

XJa Series Transformation Protocol:

1. Before starting, pre-warm plates at 37°C one for each transformation.
2. Thaw Z-Competent cells on ice.
3. Add 1-5 ul DNA and gently mix (insure that the DNA volume in less than 5% volume of the competent cells).
4. Incubate on ice for 10-20 minutes.
5. Spread 50-100 ul on a pre-warmed plate or if using a non-lactamase resistance marker, follow steps 6 - 8.
6. Add 1ml of SOC to the transformation and incubate for 1 hour at 37°C with shaking at 200-300RPM or without shaking.
7. Spread enough out growth on an appropriate selective media plate for desired number of transformants per plate.
8. Incubate the plate at 37°C or other appropriate temperature for colonies to grow.

It is important to keep in mind that chemically competent cells are extremely sensitive to changes in temperature. Transformation should be done immediately following the thawing of cells on ice. The tubes should never be exposed to room temperature for more than few seconds. Gently mix the cells by tapping with your fingers, and NOT by pipetting. Then shake the tube down in one single motion so the suspension will be collected at bottom of tube. Place it on ice or ice water bath (0°C).

XJb Series Transformation Protocol:

1. Before starting, pre-warm plates at 37°C one for each transformation.
2. Thaw Z-Competent cells on ice.
3. Add 1-5 ul DNA and gently mix (insure that the DNA volume in less than 5% volume of the competent cells).
4. Incubate on ice for 10-20 minutes.
   Note: at this point the cells can be spread directly on to an antibiotic selective plate or if a higher transformation efficiency is desired a heat shock and out growth can be performed following the procedure below.
5. Heat-shock the transformations by placing the tubes in a 42°C water bath for 15 seconds.
6. Place on ice 2 minutes.
7. Add 1ml of SOC to the transformation and incubate for 1 hour at 37°C with shaking at 200-300RPM or without shaking.
8. Spread enough out growth on an appropriate selective media plate for desired number of transformants per plate.

9. Incubate the plate at 37°C or other appropriate temperature for colonies to grow.

It is important to keep in mind that chemically competent cells are extremely sensitive to changes in temperature. Transformation should be done immediately following the thawing of cells on ice. The tubes should never be exposed to room temperature for more than few seconds. Gently mix the cells by tapping with your fingers, and NOT by pipetting. Then shake the tube down in one single motion so the suspension will be collected at bottom of tube. Place it on ice or ice water bath (0°C).

TIPS FOR QUICK TRANSFORMATION: If your experiment does not require very high transformation efficiency (e.g. when using plasmid stock to transform *E. coli*), incubate the DNA and cells on ice for 1-5 minutes and spread directly onto pre-warmed plates.

B: Autolysis of the XJ strains

The autolysis procedure can be used for extraction of intracellular material, including proteins, nucleic acids, or any other components. The protocol is designed for 2 ml final culture volume and can be modified proportionally according to your needs. Antibiotics are used when required for plasmid selection. Chloramphenicol can not be used for selection as the XJ Autolysis™ cells contain a chloramphenicol acetyltransferase (*cat*) gene inserted on the chromosome. Cells are generally grown at 37°C but lower temperatures can be used as well.

1. Cells at any stage can be used to prepare starter culture. To withdraw cells from the glycerol stock vial, remove a little of the material from the top of the frozen culture and return the tube back in the freezer promptly. Streak the cells on LB agar plates and incubate overnight. Please read Notes for Optimal Autolysis on page 5 to optimize your autolysis.

2. Prepare a starter culture by inoculating one bacterial colony into 1 ml of Luria Broth (LB) and grow for 16 hours (overnight).
   Note: Other media can be used as well. LB, and the EB/OB media, were thoroughly tested (see Dual Media Set™, Zymo Research product number M3011).

3. Add 100 ul of saturated starter culture into final 2 ml of LB broth. Add 4 ul (1/500 volume) of supplied arabinose solution. Grow cells as needed.
   Note: If the supplied arabinose solution is not available, add sterilized L-arabinose aqueous solution to final concentration of 3 mM.

4. Harvest cells and resuspend in 500 ul of buffer of your choice. See Notes for Optimal Autolysis #2 on page 5 for a recommendation for the resuspension buffer.
   Note: XJb lysis efficiency is 10-20 % lower compared to XJa. To achieve optimal lysis, more care needs to be taken when selecting a lysis buffer.

5. Freeze and thaw. Cells will be lysed at this point. A centrifugation step can be used to obtain a cell free extract. Please read Notes for Optimal Autolysis to improve the autolysis of your cells.
   Note: There are various methods to speed up the freeze-thaw process. Dry ice/ethanol bath or ultra-cold isopropanol bath will freeze the sample within seconds. A water bath of 10°C to 37°C can be used to thaw the samples quickly.

APPENDIX

Notes for Higher Z-competent Transformation Efficiency

1. **Incubation time**
   For standard transformation, incubation on ice for 10 minutes is good. This step can vary according to your needs. See the transformation time course in Figure 2.

2. **Prewarm agar plates**
   Cold plates dramatically decreased the transformation efficiency. It is strongly recommended that agar plates are prewarmed at 37°C or at least prewarmed over 20°C for this procedure.

3. **Addition of SOC**
   To increase the transformation efficiency by 2-3 fold, perform an SOC outgrowth step. Add 4 volumes of SOC (400 ul of SOC to 100 μl of transformation mixture) after incubation on ice and incubate for 1 hour at 37°C with

shaking at 200-300RPM or without shaking. Then spread the mixture directly onto prewarmed plates. In most situations, this step is not needed when ampicillin selection marker is used. When selection markers of kanamycin or tetracycline are used, this recovery step is highly recommended for efficient transformation. Reducing reagents, such as DTT (Dithiothreitol) and 2-ME(β-mercaptoethanol), are not needed for this procedure.

4. Culture conditions

The *E. coli* cells are more competent when the culture is grown at 20-25°C. Higher temperatures, such as 30°C or 37°C, decrease the transformation efficiency by 2-10 fold (see page 2). Also cells can be harvested at lower density such as OD_{600} 0.2-0.4 and resuspended at smaller volume such as 1-3 ml instead of 5 ml as recommended in the standard procedure. Cells harvested at lower density (OD600 0.2-0.6) are usually more competent than cells harvested at higher density (OD_{600}>0.6).

Notes for Optimal Autolysis

1. **Starter culture** – For best results, cells should not be growing actively prior to arabinose induction. This is achieved by using an overnight starter, where cells are already in the stationary growth phase, as directed above. If a fresher starter needs to be used, include arabinose already in the starter culture.

2. **Resuspension Buffer** – Resuspend the cell pellet in water with or without 0.01% - 0.1% Triton X-100. For His-tag purification, resuspend in the His-Binding Buffer of the His-spin Protein Miniprep kit (Zymo Research product # P2001 or P2002). Acidic buffers and buffers containing higher concentrations of Mg^{++} (>1 mM), and related metals that stabilize cell walls, inhibit lysis reaction to a various extent. If possible, add magnesium to the buffer after cells are lysed.

3. **Kinetics** - If the results obtained are not satisfactory, lysis can be significantly improved by incubating the cells at higher temperatures (25 - 37°C) or for longer time (10 or 20 minutes) after thawing (step 5).

4. **Viscosity** - Depending on the amount of material used, the lysed material may become viscous, preventing efficient manipulation. However, for most applications it is not necessary to use a large amount of cell material. If necessary, vortexing vigorously for 30 seconds will decrease viscosity in most cases. Alternatively, a nuclease treatment (e.g. DNAse I) can be used to reduce viscosity. Diluting the cell lysate with additional buffer will also reduce viscosity issues.

5. **Glycerol** – Do not perform the freezer and thaw cycle in a buffer containing glycerol. Glycerol protects the E.coli from forming ice crystals which are essential to the lysis of the cells.

6. **Glucose** – When glucose is added to the growth media, it inhibits the induction of the autolysis genes when it is present in the media. As the cells grow they consume the glucose as a carbon source. Once the glucose has been consumed autolysis begins.

7. **Chitin** – Non-λ lysozyme usually is able to degrade chitin. However, the λlysozyme expressed in these cells is not able to degrade chitin. λlysozyme is a transglycosylase.

Strain Genotypes

XJa Autolysis™ *E. coli* K recA1 supE44 endA1 hsdR17 (r_k^-, m_k^+) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB::λR,cat F'[traD36 $proAB^+$ $lacI^q$ lacZΔM15]

XJa(DE3) Autolysis™ *E. coli* K recA1 supE44 endA1 hsdR17 (r_k^-, m_k^+) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB::λR,cat F'[traD36 $proAB^+$ $lacI^q$ lacZΔM15] λDE3

XJb Autolysis™ *E. coli* B F^- ompT $hsdS_B(r_B^- m_B^-)$ gal dcm^+ araB::R,cat

XJb(DE3) Autolysis™ *E. coli* B F^- ompT $hsdS_B(r_B^- m_B^-)$ gal dcm^+ araB::R,cat λDE3

Buffers and Media

1. Arabinose Inducer Reagent

Sterile, ready-to-use.

1.5M L-arabinose
0.5M magnesium chloride

2. SOB Recipe (1 Liter):

Dissolve the following ingredients in 1 liter distilled water:

20 g tryptone	0.58 g NaCl (or 2 ml of 5M NaCl)	10 ml 1M $MgCl_2$
5 g Yeast extract	0.18 g KCl (or 0.5 ml 1M KCl)	10 ml 1M $MgSO_4$

Adjust pH to 6.0-7.0 with NaOH (about 2-3 pellets per liter). Autoclave at 10 psi for 15-20 minutes.

3. SOC Recipe (per 100ml)

Add 1 ml of a filter-sterilized 2 M glucose solution, or 2 ml of 20% (w/v) solution, to 100 ml of SOB medium.

4. LB Agar Recipe (1 Liter)

10 g of tryptone	10 g of NaCl
5 g of yeast extract	15g of agar

Adjust pH to 7.0 with sodium hydroxide. Autoclave at 15 psi for 15-20 minutes.

RELATED PRODUCTS

Product	Description	Format and Size	Catalog number
His-Spin Protein Miniprep™	Affinity purification of His-tagged proteins using Zymo's ultra-fast 5 minute formula.	**10 purifications** **50 purifications**	**P2001** **P2002**
Dual Media Set™	Set of two complementary media for recombinant protein overexpression in *E. coli*. Each set contains: 100 ml Expansion Broth™ (EB) and 500 ml Overexpression Broth™ (OB).	**100 ml EB™ &** **500 ml OB™**	**M3011**
Z-Competent *E. coli* Transformation Kit™	Zymo Research's own new method to make competent *E. coli* cells for simple and highly efficient *E. coli* transformation. Supplied with SOB.	**1 kit; yields 20 ml of competent cells**	**T3001**
Z-Competent *E. coli* Transformation Buffer Set™	Zymo Research's own new method to make competent *E. coli* cells for simple and highly efficient *E. coli* transformation. Supplied without SOB.	**1 kit; yields 60 ml of competent cells**	**T3002**

LICENSING INFORMATION

XJ Autolysis Strains

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