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

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

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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

Product	Description	Format & Size	Catalog Number	Price
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**	**$ 92.00**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3021**	**$ 184.00**
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**	**$ 92.00**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3031**	**$ 184.00**
XJb Autolysis™	*E. coli* BL21 with chromosomally inserted λ lysozyme gene inducible by arabinose.	**1 glycerol stock 1 ml 500x Arabinose**	**T5041**	**$ 92.00**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3041**	**$ 184.00**
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**	**$ 92.00**
		10 X 100 ul Z-competent cells 1 ml 500x Arabinose	**T3051**	**$ 184.00**

Reordering

Product	Description	Format & Size	Catalog Number	Price
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**	**$ 8.00**
		10 X 1 ml	**A2001-10**	**$ 52.00**

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.

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.

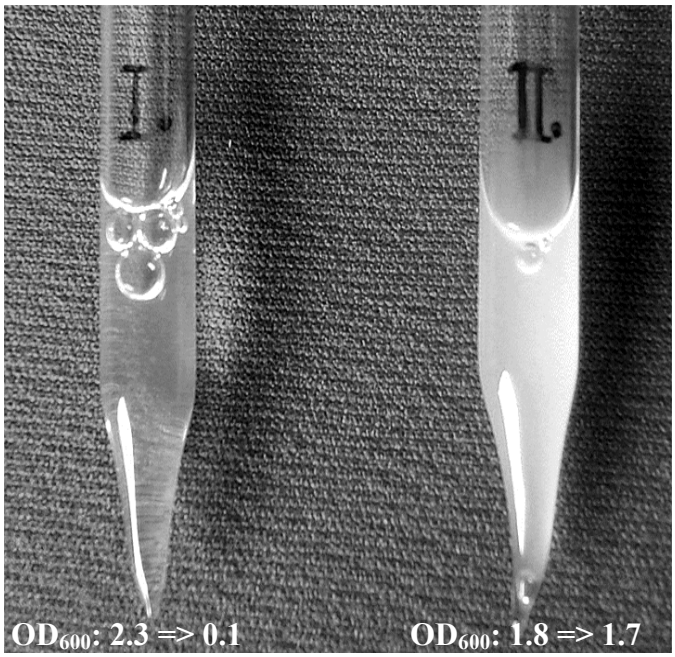
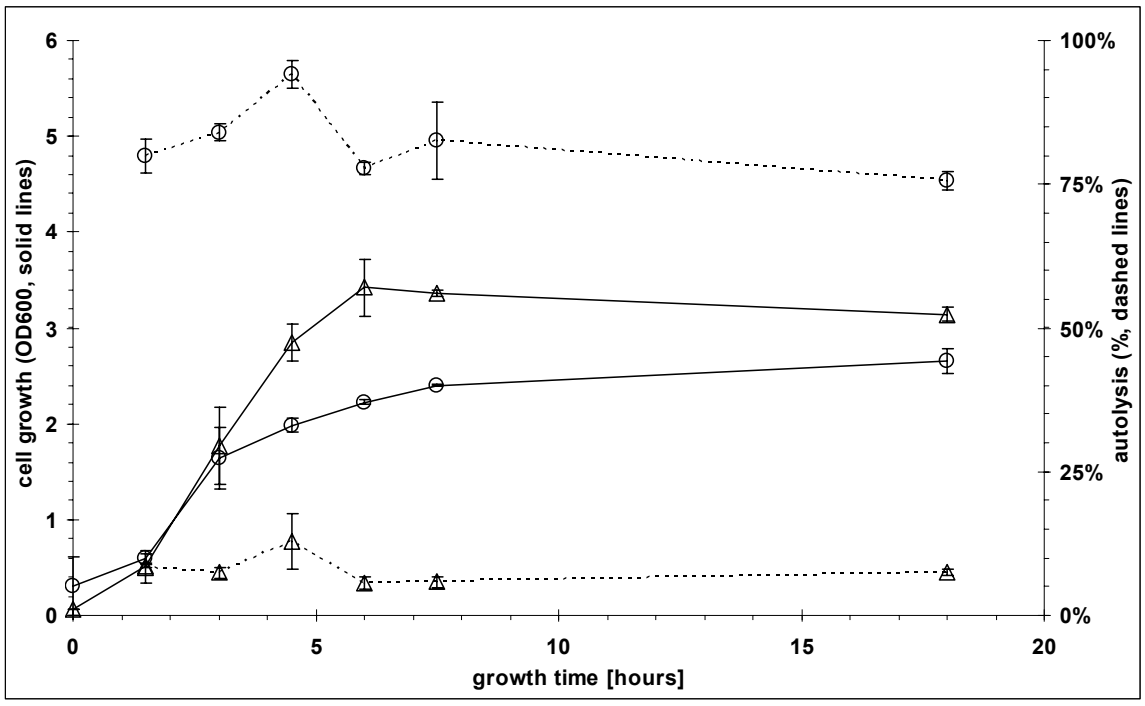


Figure 1. Autolysis of XJa cells. *E. coli* XJa cells (I) and control *E. coli* JM109 cells (II) were grown in LB for 24 hours. Autolysis was induced by arabinose addition. 4 mg of wet cells were resuspended in 1 ml of water, frozen on dry ice, and then incubated for 5 minutes on a 15°C water bath. The 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

1. Before starting, pre-warm plates at 37°C.

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.

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

Note: The procedure above is for plasmids with an ampicillin resistant marker. When kanamycin, tetracycline, erythromycin and any non-lactamase selection markers are used, an outgrowth step is needed before spreading on the plate (see page 5 for Outgrowth).

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. For transformation of Z-competent cells see **Protocol A**.

2. Prepare starter culture by inoculating one bacterial colony into 1 ml of Luria Broth (LB) and growing for 16 hours (overnight).

   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 µl of saturated starter culture into final 2 ml of LB broth. Add 4 µl (1/500 volume) of supplied arabinose solution. Grow cells as needed.

   If the supplied arabinose solution is not available, add sterilized L-arabinose aqueous solution to final concentration of 3 mM.
   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.

4. Harvest cells and resuspend in 500 µl of buffer of your choice.

   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.
   XJb lysis efficiency is 10-20 % lower compared to XJa. To achieve optimal lysis, more care needs to be taken when selecting lysis buffer. If the results obtained are not satisfactory, lysis can be significantly improved by inclusion as little as 0.01% Triton X-100 in the buffer. Lysis will also improve by incubating the cells at higher temperatures (25 - 37°C) or for longer time (10 or 20 minutes) after thawing (step 5).
   The His-Binding Buffer of the His-spin Protein Miniprep kit (Zymo Research product # P2001 or P2002) can also be used to resuspend the cell pellet.

5. Freeze and thaw. Cells will be lysed at this point. A centrifugation step can be used to obtain a cell free extract.

   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.
   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.

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

Appendix

Note for High Efficiency *E. coli* Transformation

1. Pre-warm Agar Plates

Use of cold plates will dramatically decrease the transformation efficiency. It is strongly recommended that agar plates are prewarmed at 37°C, or at least more than 20°C.

2. Incubation Time
For optimal transformation, incubation on ice should be carried out for 10 to 20 minutes; longer incubation up to 50-60 minutes acceptable. Extended incubation over 2-4 hours gradually reduces the transformation efficiency.

3. Outgrowth (addition of SOC)
When kanamycin, tetracycline, chloramphenicol, erythromycin and other non-lactamase selection markers are used, an outgrowth step is needed for efficient transformation: after incubation on ice, add 1 ml of SOC to the transformation mixture and incubate for 1 hour at 37°C before spreading onto plates. In most situations, this outgrowth is not needed when ampicillin selection marker is used. Reducing reagents, such as dithiothreitol and β-mercaptoethanol, are not needed for this procedure.

Arabinose Inducer Reagent: contains 1.5 M L-arabinose and 0.5 M magnesium chloride. Sterile, ready to use.

SOB Recipe (1 Liter):

Dissolve the following ingredients in 1 liter distilled water:

20 g Bacto-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.

SOC Formula (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.

LB Agar (1 Liter)

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

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

Related Products

Product	Description	Format & Size	Catalog Number	Price
His-Spin Protein Miniprep™	Affinity purification of His-tagged proteins using Zymo's ultra-fast 5-minute formula.	**10 purifications 50 purifications**	**P2001 P2002**	**$ 62.00 $245.00**
Dual Media Set™	Set of two complementary media for recombinant protein overexpression in *E. coli*. Each set contains: 100 ml Expansion Broth™ (EB) and 500 ml Overexpression Broth™ (OB).	**100 ml EB™ & 500 ml OB™**	**M3011**	**$ 38.00**
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**	**$92.00**
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**	**$99.00**