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ZYMO RESEARCH

DNA
Purification
MADE SIMPLE
Made Simple™

XJ Autolysis™ *E. coli* Strains

For the simple, efficient, and gentle lysis of *E. coli* cells.

Highlights

- Simple and controlled autolysis of *E. coli*.
- Strains can be 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.

Catalog Numbers:

T5021, T5031, T5041, T5051, T3021, T3031, T3041, T3051



Scan with your smart-phone camera to view the online protocol/video.



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Product Contents

	T5021, T5031, T5041, T5051	T3021, T3031, T3041, T3051
<i>Mix & Go!</i> Competent Cells	-	10 x 100 μ l Single – Tube Aliquots 1 ml 500x Arabinose
Glycerol Stock	1 Glycerol Stock 1 ml 500x Arabinose	-
Instruction Manual	1	1

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.

Mix & Go! 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.

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.

Product 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 autolyzing 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 peptidoglycan 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 (Figure 1). 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.

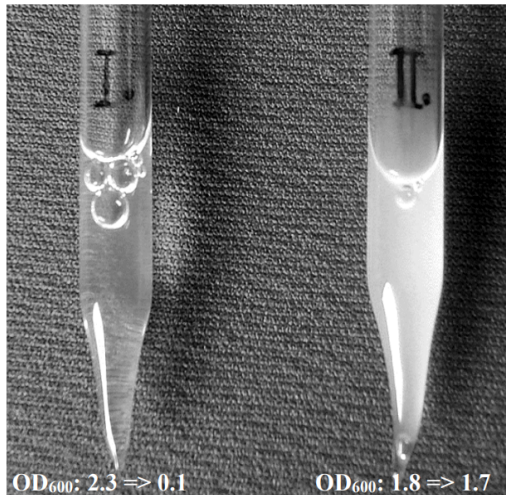
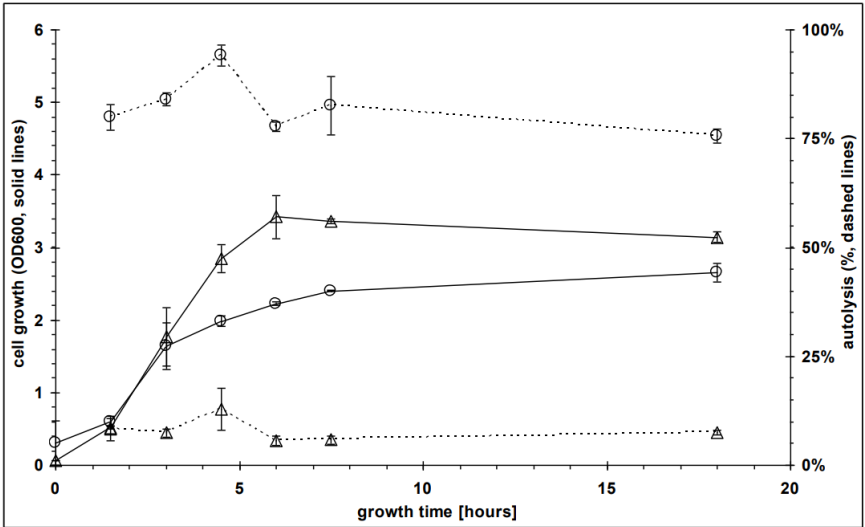


Figure 1. Autolysis of XJa cells. *E. coli* XJa cells (I) and control *E. coli* JM109 cells (II) were grown in LB for 24 hours. Autolysis was induced by arabinose addition. 4 mg of wet cells were resuspended in 1 ml of water, frozen on dry ice, and then incubated for 5 minutes on a 15°C water bath. The OD₆₀₀ 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).

Protocol

A. Transformation of *Mix & Go!* Competent Cells

The *Mix & Go!* *E. coli* cells are pre-made chemically competent cells for simple and highly efficient *E. coli* transformation. *Mix & Go!* *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 *Mix & Go!* cells, incubate on ice for 10-20 minutes, and spread on plates. The pre-made *Mix & Go!* 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 *Mix & Go!* competent cells are supplied as a pack of 10 convenient 100 μl /tube single use aliquots.

XJa Series Transformation Protocol:

1. Before starting, prewarm plates at 37°C one for each transformation.
2. Thaw *Mix & Go!* competent cells on ice.
3. Add 1-5 μl DNA and gently mix (ensure that the DNA volume is less than 5% volume of the competent cells).
4. Incubate on ice for 10-20 minutes.
5. Spread 50-100 μl on a prewarmed 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 200-300 RPM or without shaking.
7. Spread enough outgrowth 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.

Note: 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, prewarm plates at 37°C one for each transformation.
2. Thaw *Mix & Go!* competent cells on ice.
3. Add 1-5 ul DNA and gently mix (ensure that the DNA volume is 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 outgrowth 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 200-300 RPM or without shaking.
8. Spread enough outgrowth 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.

Note: 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 prewarmed 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 cannot 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 9 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™, Cat. No. 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.

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 µl of buffer of your choice. See Notes for Optimal Autolysis #2 on page 9 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 *Mix & Go!* Competent Cells 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 decrease 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 µl of SOC to 100 µl of transformation mixture) after incubation on ice and incubate for 1 hour at 37°C with shaking at 200-300 RPM 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 3). Also, cells can be harvested at lower density such as OD₆₀₀ 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 (OD₆₀₀>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 (Cat No. 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

Usually, non- λ lysozyme is able to degrade chitin. However, the λ lysozyme expressed in these cells is not able to degrade chitin. λ lysozyme is a transglycosylase.

Strain Genotypes

XJa
Autolysis™ F⁻[traD36 proA⁺B⁺ lacI^q Δ (lacZ)M15] Δ (lac-proAB) glnV44 (supE44) e14⁻ (McrA⁻) thi gyrA96 (Nal^R) endA1 hsdR17(r_K⁻ m_K⁺) relA1 recA1 Δ araB:: λ R, cat (Cm^R)

XJa(DE3)
Autolysis™ F⁻[traD36 proA⁺B⁺ lacI^q Δ (lacZ)M15] Δ (lac-proAB) glnV44 (supE44) e14⁻ (McrA⁻) thi gyrA96 (Nal^R) endA1 hsdR17 (r_K⁻ m_K⁺) relA1 recA1 Δ araB:: λ R, cat (Cm^R), λ (DE3)

XJb
Autolysis™ F⁻ ompT hsdS_B(r_B⁻ m_B⁻) gal dcm⁺ Δ araB:: λ R,cat (Cm^R)

XJb(DE3)
Autolysis™ F⁻ ompT hsdS_B(r_B⁻ m_B⁻) gal dcm⁺ Δ araB:: λ R, cat (Cm^R), λ (DE3)

Buffer 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 MgCl₂

5 g Yeast extract

0.18 g KCl (or 0.5 ml 1M KCl)

10 ml 1M MgSO₄

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.

Licensing Information

XJ Autolysis Strains

The XJ Autolysis strains are based on technology developed by Zymo Research Corp. and are patent pending. By purchasing the XJ Autolysis strains, ZRC grants the user non-exclusive licensing for the use of XJ Autolysis strains for research use only. No strains may be distributed further to third parties outside of the original users laboratory. Zymo Research Corporation requires additional licensing for commercial use, including the use of these materials for production purposes by any commercial entity. To obtain information on additional commercial licensing please contact the Office of Intellectual Property at licensing@zymoresearch.com or call 888-882-9682.

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T7 Expression System

The T7 expression system is based on technology developed by Brookhaven National Laboratory under contract with the U.S. Department of Energy and is subject to protection by U.S. patents and patent applications assigned to Brookhaven Science Associates, LLC. The terms of licensing are provided for research use only. To protect its patent properties, Brookhaven Science Associates requires commercial entities doing business in the United States, and its Territories or Possessions to obtain the proper licensing to use the technology for commercial use.

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Ordering Information

Product	Description	Catalog No.	Format and Size
XJa Autolysis™	E. coli JM109 with chromosomally inserted λ lysozyme gene inducible by arabinose.	T5021	1 glycerol stock 1 ml 500x Arabinose
		T3021	10 X 100 μ l Mix & Go cells* 1 ml 500x Arabinose
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.	T5031	1 glycerol stock 1 ml 500x Arabinose
		T3031	10 X 100 μ l Mix & Go cells* 1 ml 500x Arabinose
XJb Autolysis™	E. coli BL21 with chromosomally inserted λ lysozyme gene inducible by arabinose.	T5041	1 glycerol stock 1 ml 500x Arabinose
		T3041	10 X 100 μ l Mix & Go cells* 1 ml 500x Arabinose
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.	T5051	1 glycerol stock 1 ml 500x Arabinose
		T3051	10 X 100 μ l Mix & Go cells* 1 ml 500x Arabinose

* Mix & Go cells are chemically competent cells with high transformation efficiency.

Individual Kit Components	Description	Catalog No.	Size
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)	A2001-1	1 X 1 ml
		A2001-10	10 X 1 ml



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