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BL21-AI™ One Shot® Chemically Competent *E. coli*

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User Manual

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About the Kit

Shipping/ Storage

The BL21-AI™ One Shot® Chemically Competent *E. coli* Kit is shipped on dry ice. Upon receipt, store at –80°C.

Kit Contents

The table below describes the items included in the BL21-AI™ One Shot® Chemically Competent *E. coli* Kit. The transformation efficiency of BL21-AI™ cells is 1 x 10^8 cfu/µg of pUC18 plasmid DNA.

Store at –80°C.

Item	Composition	Amount
SOC Medium (store at room temperature or 4°C)	2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose	6 mL
20% L-arabinose	20% L-arabinose in sterile water	1 mL
BL21-AI™ chemically competent cells	—	21 × 50 µL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µL

Genotype

BL21-AI™: F$^-$ *ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm araB*::*T7RNAP-tetA*

The BL21-AI™ strain is an *E. coli* B/r strain and does not contain the *lon* protease. It is also deficient in the outer membrane protease, OmpT. The lack of these proteases reduces degradation of heterologous proteins expressed in this strain.

The strain carries a chromosomal insertion of a cassette containing the T7 RNA polymerase (T7 RNAP) gene in the *ara*B locus, allowing expression of T7 RNAP to be regulated by the *araBAD* promoter (see page 1 for more information).

The presence of the *tet*A gene confers resistance to tetracycline and permits verification of strain identity using tetracycline.

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About the Kit, Continued

The BL21-AI™ Strain

The BL21-AI™ *E. coli* strain is derived from the BL21 strain (Grodberg and Dunn, 1988; Studier and Moffatt, 1986) and is intended for use as an expression host. The BL21-AI™ strain contains a chromosomal insertion of the gene encoding T7 RNA polymerase (T7 RNAP) into the *ara*B locus of the *araBAD* operon, placing regulation of T7 RNAP under the control of the *araBAD* promoter. The *ara*B gene is deleted in this strain.

Regulating Expression of T7 RNA Polymerase

Because the T7 RNAP gene is inserted into the *ara*B locus of the *araBAD* operon, expression of T7 RNA polymerase can be regulated by the sugars, L-arabinose and glucose.

- To induce expression from the *araBAD* promoter, use L-arabinose (Lee, 1980; Lee *et al.*, 1987). To modulate expression, simply vary the concentration of L-arabinose added.
- To repress basal expression from the *araBAD* promoter, use glucose.

  Note: In the absence of glucose, basal expression from the *araBAD* promoter is generally low (Lee, 1980; Lee *et al.*, 1987). Adding glucose further represses expression from the *araBAD* promoter by reducing the levels of 3´, 5´-cyclic AMP (Miyada *et al.*, 1984).

For more information on the mechanism of expression and repression of the *ara* regulon, see page 11 or refer to Schleif, 1992.

Expression of Heterologous Genes

The BL21-AI™ strain is suitable for high-level recombinant protein expression from any T7-based expression vector. Because T7 RNA polymerase levels can be tightly regulated, the BL21-AI™ strain is especially useful to express genes that may be toxic to other BL21 strains where basal expression of T7 RNA polymerase is leakier (*e.g.* BL21 Star™(DE3) or BL21(DE3)). The yield of recombinant protein obtained from BL21-AI™ is generally similar to that obtained from other BL21 strains.

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About the Kit, Continued

When to Use BL21-AI™

In general, we recommend using the BL21-AI™ strain to express your gene of interest if:

- You are using a T7-based expression vector (either high-copy or low-copy)
- You observe growth inhibitory effects (i.e. toxicity) when using other BL21 strains (see page 8 for more information about indications of toxicity in bacterial cells)

You are expressing a known toxic gene

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Basic Transformation Procedure

Introduction

A basic transformation protocol for BL21-AI™ cells is provided below. Once you have selected transformants, we recommend proceeding directly to expression using your own protocol. Note that the BL21-AI™ strain is designed for expression, not cloning or subcloning.

Materials Supplied by the User

- Plasmid DNA (ready for transformation)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- Ice bucket with ice
- Spectrophotometer to measure optical density of the cell cultures
- Microcentrifuge tube rack (optional)

Before Starting

- Prepare LB agar plates containing the appropriate concentration of antibiotic for plasmid selection
- Equilibrate a water bath to 42°C
- Warm the vial of SOC medium to room temperature
- Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation)

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Basic Transformation Procedure, Continued

Basic Transformation Procedure

1. Thaw one vial of BL21-AI™ One Shot® cells on ice per transformation.
2. Add 5–10 ng of DNA, in a volume of 1–5 µL to the cells and mix by tapping gently. **Do not mix cells by pipetting.**
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat shock the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. **Do not mix or shake.**
5. Remove the vial(s) from the 42°C bath and quickly place on ice.
6. Add **250 µL** of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; use proper sterile technique to avoid contamination.)
7. Secure the vial(s) in a microcentrifuge rack with tape. Place the rack in a shaking incubator, and shake the vial(s) at 37°C for 1 hour at 225 rpm.
8. Plate two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Include 34 µg/mL chloramphenicol if using BL21(DE3)pLysS or BL21(DE3)pLysE cells. Select two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. The remaining transformation reaction may be stored at 4°C and plated out the next day, if needed.
9. Invert the plates and incubate at 37°C overnight.
10. Select transformants from the plates and culture as described on page 7.

    Note: Clones may exhibit differences in expression of heterologous genes. We recommend choosing 3–4 transformants when characterizing clones for protein expression.

Expression Guidelines

Introduction

If you have an expression protocol for the plasmid that you are working with, we recommend that you use your own protocol. This section provides some general guidelines for the use of T7 RNA polymerase-based expression plasmids in BL21-AI™ cells.

Use BL21-AI™ cells **for expression only**. To maintain your construct, transform your expression plasmid into a strain that does not bear the gene for T7 RNA polymerase (i.e. TOP10, DH5α™).

Modulating Gene Expression

To induce expression of your gene of interest in BL21-AI™ cells, you will need the following reagents:

- L-arabinose to induce expression of T7 RNA polymerase. L-arabinose is supplied with the kit, but is also available from Sigma (Catalog no. A3256).
- Isopropyl β-D-thiogalactoside (IPTG; only required for T7-based expression vectors containing the *lac*I gene). IPTG is available from Invitrogen (Catalog no. 15529-019).

Note: You may repress **basal** transcription of T7 RNA polymerase and thereby, your gene of interest by adding glucose to the plates and/or media (see pages 8 and 11 for more information). By varying the concentration of L-arabinose and glucose, you may modulate expression of your gene of interest.

Materials Needed

You should have the following stock solutions on hand before beginning. Prepare all solutions in sterile, deionized water.

- 20% L-arabinose (supplied with the kit)
- 1 M IPTG (if needed)
- 20% glucose (if needed)

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Expression Guidelines, Continued

Expression Guidelines

General guidelines are provided below to express your gene of interest in BL21-AI™.

- Pick 3–4 transformants for overnight culture in 5 mL LB medium containing antibiotic to select for your expression plasmid. Grow overnight at 37°C with shaking until the OD_{600} reaches 0.6–1.0.
- Use the overnight cultures to inoculate fresh LB medium containing antibiotic to an OD_{600} of 0.05–0.1 (~1:20 dilution of the overnight culture). This dilution allows the cells to quickly return to logarithmic growth and reach the appropriate cell density. Use a volume appropriate for taking time points, if desired.
- Use the remainder of each overnight culture to create glycerol stocks. Once you have identified the clone that best expresses your protein, you can use the glycerol stock to perform additional expression experiments.
- Grow the cultures until they reach mid-log phase (OD_{600} ~0.4; 2 to 3 hours).
- Induce the cultures (see below), and culture for an additional 2–3 hours. You may also take time points to analyze for optimal expression of your protein.

  For T7 expression vector containing the *lac*I gene (e.g. Invitrogen's pET vectors), induce by adding L-arabinose to a final concentration of 0.2% **AND** IPTG to a final concentration of 1 mM.

  For T7 expression vector with no *lac*I gene (e.g. Invitrogen's pCR®T7 vectors), induce by adding L-arabinose to a final concentration of 0.2%. Culture for an additional 2–3 hours.
- Analyze clones by western blot or enzymatic assay to determine which clone best expresses your protein of interest. Use the glycerol stock created from this clone for expression experiments. If you find that expression levels in subsequent inductions decrease, or you find that you lose your plasmid, your protein may be toxic to *E. coli* (see page 8 for additional information).

Continued on next page

Expression Guidelines, Continued

Indications of Toxicity

When expressing recombinant proteins in BL21-AI™ strains, one can generally assume that the recombinant protein is toxic to bacterial cells when any of the following occurs:

- No transformants are obtained after following Steps 1-9 of the **Basic Transformation Protocol** on page 5 **OR** a combination of large and small, irregular colonies appears on the plate
- The initial culture does not grow (see previous page)
- It takes longer than 5 hours after a 1:20 dilution of the initial culture for the fresh culture to reach an OD_{600}=0.4 (see previous page)
- The cells lyse after induction with L-arabinose (or L-arabinose and IPTG) (see previous page)

Precautions

Review the guidelines below when basal level expression of a gene of interest is toxic. These guidelines assume that the T7 expression plasmid has been correctly designed and created.

- Propagate and maintain your expression plasmid in a strain that does not contain T7 RNA polymerase (i.e. TOP10, DH5α™, etc.).
- Perform a fresh transformation of BL21-AI™ cells before each induction experiment.
- Minimize the amount of time that the cells bearing the gene of interest are cultured before induction.
- Supplement LB plates in **Basic Transformation Protocol** (page 5 Step 7), and growth medium for expression experiments (page 7) with 0.1% glucose. The presence of glucose represses basal expression of T7 RNA polymerase.
- Following transformation of BL21-AI™ cells, grow cells in SOC medium for 1 hour and go directly to protein expression. Do not plate the transformation mixture to select for individual clones. See next page for details.

Continued on next page

Expression Guidelines, Continued

Transformation/ Expression Protocol for Toxic Genes

This protocol is used with BL21-AI™ cells. Please note that other protocols are possible, depending on your needs.

Transformation

1. Follow the basic transformation protocol on page 5 through Step 7.
2. After growing the transformation reaction in SOC for 1 hour (page 5, Step 7), add the **entire** transformation reaction (300 µL) to 50–200 mL of LB medium pre-warmed to 37°C containing the appropriate selective antibiotic for your expression plasmid (and 0.1% glucose, if desired).

Induction

3. Incubate the vial(s) with shaking at 37°C until the cells reach mid-log phase (OD_{600} = 0.4). **Note**: Doubling times may vary (30 to 90 minutes) depending on the protein expressed.
4. Induce expression of the recombinant protein by adding L-arabinose to a final concentration of 0.2% (and IPTG to a final concentration of 1 mM, if needed) and grow for 2–3 more hours. You make take time points, if desired.
5. Harvest cells by centrifugation and use immediately for analysis, or store the cell pellet at –80°C.

Testing Transformation Efficiency

Introduction

To test the transformation efficiency of the competent cells contained in the One Shot® kit, use the supercoiled pUC19 plasmid supplied with the kit as described below. An extra vial of cells is included for this purpose.

Before Starting

- Prepare LB agar plates containing 50 µg/mL ampicillin.
- Equilibrate a water bath to 42°C.
- Warm the vial of SOC medium to room temperature.
- Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation).

Transformation

Follow the transformation protocol on page 5 to transform pUC19 into BL21-AI™. Use the specific modifications below.

- Transform cells with 1 µL (10 pg) of pUC19
- Plate 50 µL each onto two LB plates containing 50 µg/mL ampicillin
- Calculate the transformation efficiency as transformants per µg of plasmid (see below). The cells should have an efficiency of 1 x 10^8 transformants/µg of supercoiled plasmid

Calculation

Use the formula below to calculate transformation efficiency.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µL transformed cells}}{\text{X µL plated}} = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

Regulation by L-Arabinose

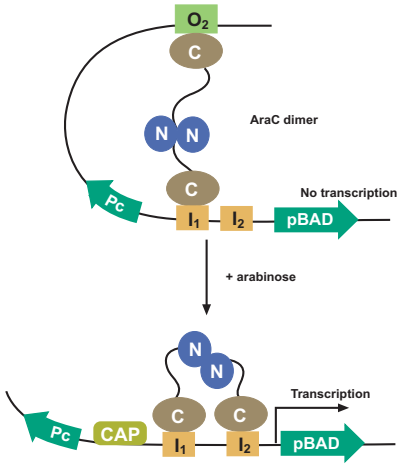
Introduction

The L-arabinose regulatory circuit is briefly described below.

Regulation of the *araBAD* (P_{BAD}) Promoter

The *ara*BAD promoter (P_{BAD}) used to control expression of T7 RNA polymerase in BL21-AI™ is both positively and negatively regulated by the product of the *ara*C gene (Ogden *et al.*, 1980; Schleif, 1992). AraC is a transcriptional regulator that forms a complex with L-arabinose. In the absence of L-arabinose the AraC dimer contacts the O2 and I1 half sites of the *ara*BAD operon, forming a 210 bp DNA loop (see figure below). For maximum transcriptional activation two events are required.

- L-Arabinose binds to AraC and causes the protein to release the O2 site and bind the I2 site which is adjacent to the I1 site. This releases the DNA loop and allows transcription to begin.
- The cAMP activator protein (CAP)-cAMP complex binds to the DNA and stimulates binding of AraC to I1 and I2.



Glucose Repression

Basal expression levels can be repressed by adding glucose to the growth medium. Glucose lowers cAMP levels, which in turn decreases the binding of CAP. As cAMP levels are lowered, transcriptional activation is decreased.

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information.
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Purchaser Notification

Information for European Customers

The BL21-AI™ *E. coli* strain is genetically modified and carries a chromosomal insertion of a cassette containing the T7 RNA polymerase (T7 RNAP) gene. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Limited Use Label License No: 30 T7 Expression System

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