INSTRUCTIONS

Dual Media Set™ For Protein Expression

Expansion Broth™ (EB™) and Overexpression Broth™ (OB™)

Catalog No. M3011

Highlights

- Reliable method for high level recombinant protein expression in *E. coli*
- Simple procedure: inoculate bacteria into **EB™** medium for cell expansion, add **OB™** medium for protein over-expression
- Eliminates the need to monitor culture density and to optimize timing of inducer addition
- Synchronizes cultures expressing diverse recombinant proteins

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

ZYMO RESEARCH • 625 West Katella Avenue, Suite 32 • Orange, CA 92867-4619, USA
Toll Free: 1.888.882.9682 • Fax: 714.288.9643 • Internet: www.zymoresearch.com • E-mail: info@zymoresearch.com

GENERAL INFORMATION

Set Contents

Item	Amount	Storage
Expansion Broth™ (EB™)	100 ml	Room Temperature
Overexpression Broth™ (OB™)	500 ml	Room Temperature
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Ordering Information

Products	Cat No	Size
Dual Media Set™ For Protein Expression Contains 100 ml EB™ and 500 ml OB™	**M3011**	**1 set**
Media For Individual Sale:		
Expansion Broth™	M3012-100	100 ml
Expansion Broth™	M3012-500	500 ml
Overexpression Broth™	M3013-100	100 ml
Overexpression Broth™	M3013-500	500 ml

Overview

Although recombinant protein expression in *E. coli* has become all but routine, high level protein expression or over-expression is not always consistent and repeatable for every protein. Our research at Zymo Research Corp. has shown that high level protein expression can be achieved consistently when two processes - *cell expansion* and *protein expression* - are completely separated.

The **Dual Media Set™**, different from commonly used protein expression procedures using Luria Broth (LB) or other specially prepared medium, contains two media, **EXPANSION BROTH™** (**EB™**) and **OVEREXPRESSION BROTH™** (**OB™**). For cell expansion, *E. coli* cells are grown in **EB™**, while the production of the recombinant protein is almost completely repressed. For high level protein expression, the expanded cell culture is simply added into **OB™** media (see Figure 1). By using the **Dual Media Set™** system, protein overexpression can be reliably controlled for many proteins, as demonstrated in Figure 2.

In some circumstances, such as when expressing toxic proteins, or when the expressed protein is poorly soluble, over-expression is counter-productive. In such cases, limited protein production can be initiated by adding the specific inducer (IPTG for *lac*-based promoters) to cells growing in **EB™** (see Figure 1 and Protocol II.)

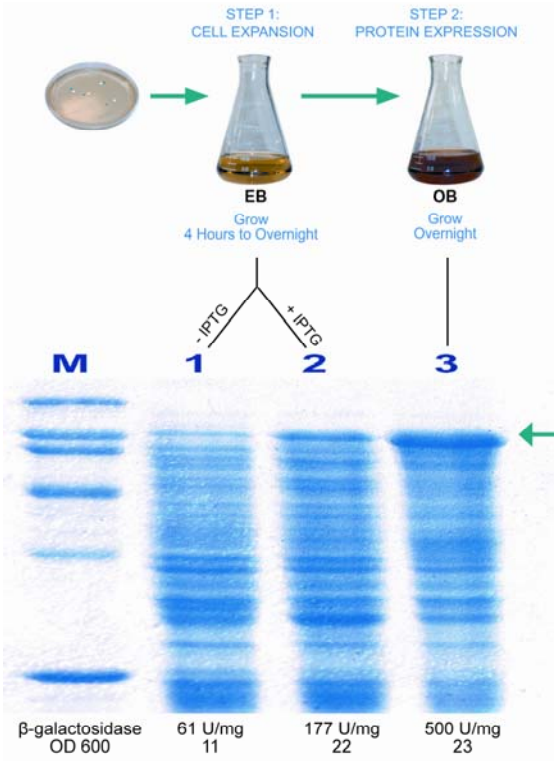

Figure 1. **Controlled overexpression of β-galactosidase.** Cell culture is first expanded in EB™, where only background levels of the T7-*lac* promoter-controlled product are produced (1). Moderate amounts of the enzyme are produced by overnight incubation in EB™ with IPTG (2), highest amounts are produced in OB™ (3).

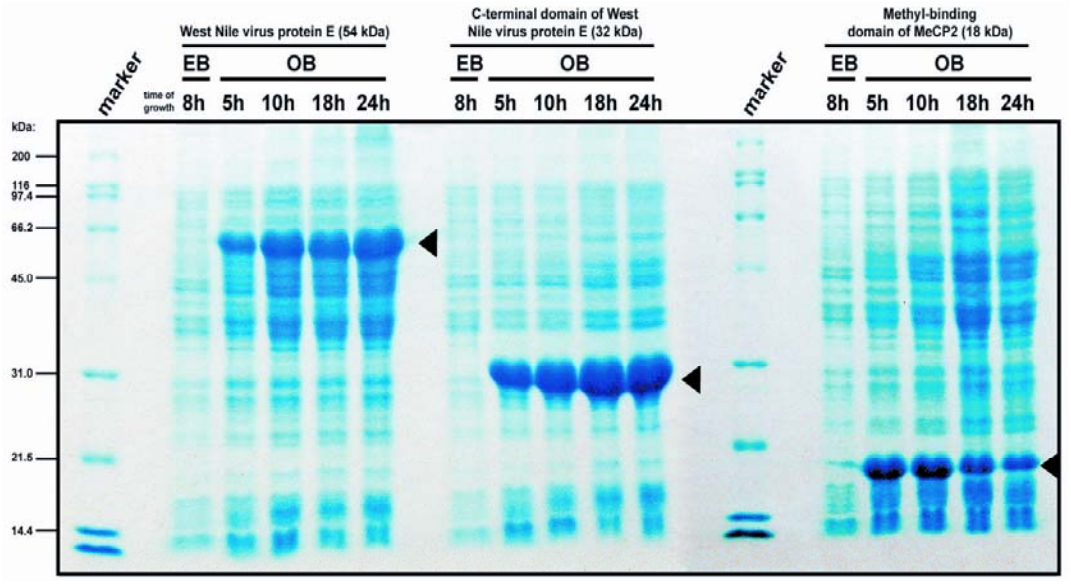

Figure 2.: **SDS-PAGE electrophoresis of cell proteins after growth in Dual Media Expression system.** The recombinant proteins (indicated by solid arrows) were expressed in *E. coli* under the T7-*lac* promoter. Expression of the recombinant protein was virtually abolished during the initial 8-hour expansion in EB™. After adding 3 volumes of OB™ to the expanded culture, the growth was continued for up to 24 additional hours, removing samples at indicated times. The cell pellet was resuspended directly in SDS-PAGE loading buffer and analyzed on a 15% polyacrylamide gel. Proteins were stained by Coomassie blue® after the electrophoresis.

Protocols

Protocol I is recommended for most protein expression experiments. **Protocol II** is designed for toxic proteins, insoluble proteins, or when low level protein expression is required. The protocols are for 20 ml final culture volume and can be modified proportionally according to your needs. Please carefully read the following notes:

1. To ensure protein/plasmid stability, use fresh bacterial colonies after plasmid transformation. This minimizes the risk of plasmid deletions or mutations, or other alterations.
2. When the protein/plasmid stability is not an issue, any source of cells can be used as the starting material.
3. To achieve best results for both repression and induction of protein expression in these media, it is very important to provide good aeration. This can be achieved by using high-speed shaker incubators (300 rpm or higher) and using the highest practically acceptable ratio of flask/culture volumes (e.g. 20/1). Baffles in flask walls, if available, also improve aeration.
4. Growth at lower temperatures for extended time (for example, 1-2 days at room temperature) further improves protein expression yields and solubility in many cases.

Please visit our website www.zymoresearch.com for additional information on using the Dual Media Set™.

Protocol I – default protocol for most protein expression experiments

1. Inoculate a freshly-growing bacterial colony into 5 ml EB™ . Grow for 4 hours to overnight (resulting OD_{600} = 5 or higher).

2. Add three volumes (15 ml) of pre-warmed OB™. Add 0.25 mM IPTG for *lac*-based promoters (*lac*, *tac*, *trc*). DO NOT add IPTG for T7-based expression systems such as in the pET plasmid series.

3. Grow overnight and harvest the cells. The resulting OD_{600} is often > 20. Optionally, samples may be removed at convenient intervals (e.g. 2 - 24 hours) to check for levels of protein expression.

Protocol II -for toxic proteins or when low level protein expression is required.

1. Inoculate a freshly-growing bacterial colony into 5 ml EB™. Grow for 4 to 8 hours.

2. Add three volumes (15 ml) of pre-warmed EB™. Add 0.25 mM IPTG and continue incubation overnight. This will result in weak-to-moderate protein expression, which can facilitate the expression of toxic, unstable, or insoluble proteins.

Frequently Asked Questions

1. What underlying mechanism ensures that the **Dual Media Set™** works so reliably for protein overexpression?

Our research has found that the use of the common Luria Broth (LB) medium produces relatively high background levels of recombinant protein expression even without the inducer (such as IPTG). The expression often does not increase enough after addition of the inducer. We have concluded that although LB is an excellent medium for normal *E. coli* manipulation, it is not a good medium for repression of protein expression during cell expansion, nor is it ideal for protein expression when inducer is added. **EB™** and **OB™** were designed to overcome these problems. **EB™** was designed to repress recombinant protein expression by regulating the levels of cyclic AMP (cAMP) and cAMP receptor protein (CRP) during cell expansion, so that the cells can be expanded without pressure of undesired foreign protein expression and unintentional selection of mutated clones to overgrow original inoculates. When the cells are expanded, **OB™** medium is added for protein expression. During this process, further cell growth is not required and selection of new mutants no longer occurs because cell replication is very limited at this stage. The carefully formulated composition of **OB™** works similarly by regulating the levels of cAMP and CRP but with an opposite effect than **EB™**. Therefore, addition of IPTG is not needed for strong promoters such as T7.

2. What are the ingredients of **EB™** and **OB™** ?

Our growth media contain only standard commonly used ingredients, salts, and carbohydrates. The final composition, and the ratio of individual components, has been carefully optimized to result in above described metabolic effects.

3. Can I replace **EB™** or **OB™** with my home made media?

EB™ can be replaced with LB when the expressed protein is stable and does not interfere with cell growth. **OB™** cannot be replaced.

4. Can I use **OB™** without the initial cell expansion in **EB™** ?

The simplified protocol can be used, but only for most stable and easily expressed proteins. You can simply start at step 2. in Protocol I. This will result in strong protein overexpression, with the risk of introducing unwanted mutations or other unwanted effects.

5. I have always used IPTG to induce protein expression in the T7 system, why not now? Will I get less protein if I don't use IPTG in such case?

The T7 promoter is too strong when induced with IPTG. The metabolic effects caused by growth in **OB™** are also strong. This is a powerful combination, always resulting in too rapid protein expression with negative effects on cell viability. This situation results in lower yields of recombinant protein and may lead to additional plasmid/protein instability.

Please visit our website www.zymoresearch.com for additional information and updated tips on using the Dual Media Set™.