Overnight Express™ Autoinduction Systems

About the System

Overnight Express Autoinduction System 1	71300-3 71300-4
Overnight Express Autoinduction System 2	71366-3 71366-4

Description

The Overnight Express Autoinduction Systems 1 and 2 are designed for high-level protein expression in complex or chemically defined media, respectively, with the pET bacterial expression system (1). In λDE3 lysogenic hosts, uninduced cells grow to a high cell density followed by automatic induction of T7 RNA polymerase expression without monitoring cell density or adding IPTG. The Overnight Express System 1 contains three components: OnEx™ Solution 1 (induction solution); OnEx Solution 2 (buffering solution); and OnEx Solution 3 (magnesium solution). OnEx Solution 1 is a blend of carbon sources optimized for tightly regulated uninduced growth to high cell density followed by high-level induction by lactose and continued growth. OnEx Solution 2 is a concentrated buffer that maintains pH throughout metabolic acid production and supplies additional nitrogen necessary to support increased protein synthesis. OnEx Solution 3 provides critical magnesium for maximum cell density. Addition of these components to traditional glucose-free complex media such as LB broth, TB, or animal-free Veggie™ medium results in maximum yields of target proteins with the pET system (2).

The Overnight Express System 2 contains the same three OnEx Solutions 1–3 plus three additional components: OnEx Solutions 4–6. OnEx Solution 4 (metals mixture) provides trace metals below toxic levels to minimize growth limitations associated with mineral deficiencies and saturates almost any metal-containing target protein even at high expression levels. OnEx Solution 5 is an amino acid mixture lacking methionine, cysteine, and tyrosine. OnEx Solution 6 (methionine solution) is an individual solution of methionine. Adding these six components to sterile water results in a defined medium capable of promoting high cell densities, enabling autoinduction of expression, producing maximum soluble protein yields, and, if desired, efficient labeling of target proteins by the addition of Se-Met (3). The Overnight Express Autoinduction Systems are extremely convenient for routine expression of proteins in multiple cultures in either complex (System 1) or defined (System 2) media and the systems are ideal for high-throughput parallel analysis of protein expression, solubility, and purification from multiple expression clones. Additionally, System 2 can be used for selenomethionyl (Se-Met) labeling of proteins to be crystallized for X-ray diffraction studies. System 2 medium contains sufficient methionine to allow faster growth by the methionine auxotroph B834 in the presence of Se-Met and will result in expression of fully labeled target proteins from either the B834 methionine auxotroph or BL21(DE3) prototroph.

The kits provide sufficient reagents for 1 liter or 5 liters of media.

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Components

Overnight Express System 1

- 20 or 100 ml OnEx Solution 1
- 50 or 2 × 125 ml OnEx Solution 2
- 1 or 5 × 1 ml OnEx Solution 3

Overnight Express System 2

- 20 or 100 ml OnEx Solution 1
- 50 or 2 × 125 ml OnEx Solution 2
- 1 or 5 × 1 ml OnEx Solution 3
- 1 or 5 × 1 ml OnEx Solution 4
- 20 or 100 ml OnEx Solution 5
- 20 or 100 ml OnEx Solution 6

Storage

Store solutions 1–4 at 4°C. Store solutions 5 and 6 at –20°C.

Notes: *OnEx Solutions 1–6 are supplied as sterile solutions and should be used employing aseptic techniques.*

If a precipitate forms in OnEx Solution 2 during storage at 4°C, incubate in a room temperature water bath with gentle swirling or inversion to redissolve.

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Overnight Express Autoinduction System 1

Culture media

Overnight Express System 1 is compatible with glucose-free media such as Terrific Broth (TB), Luria-Bertani (LB) broth and 2X YT.

Overnight Express System 1 medium
Aseptically combine the following reagents
Per liter:
20 ml OnEx™ Solution 1
50 ml OnEx Solution 2
1 ml OnEx Solution 3
929 ml sterile glucose-free medium

Some common media formulations are listed below.

LB broth	
Per liter: 10 g tryptone 5 g yeast extract 10 g NaCl • Adjust pH to 7.5 with 1 N NaOH • Adjust volume to 1 L with deionized water and autoclave	
TB	**2X YT**
Per liter: 900 ml deionized water 12 g tryptone 24 g yeast extract 4 ml glycerol • Adjust volume to 1 L with deionized water and autoclave	Per liter: 900 ml deionized water 16 g tryptone 10 g yeast extract 5g NaCl • Adjust pH to 7.0 with 5 N NaOH • Adjust volume to 1 L with deionized water and autoclave

Overnight Express Autoinduction System 2

Chemically defined culture media for autoinduction

Overnight Express System 2 medium
Aseptically combine the following reagents in order[1]
Per liter:
1 ml OnEx Solution 3
1 ml OnEx Solution 4[2]
20 ml OnEx™ Solution 1
50 ml OnEx Solution 2
20 ml OnEx Solution 5
20 ml OnEx Solution 6
888 ml sterile glucose-free medium

[1]When using a *metE* minus host strain [i.e., B834(DE3)], adding a final concentration of 100nM vitamin B12 to the medium significantly increases the yield of target protein (1).

[2]The trace metals present in OnEx Solution 4 may affect certain applications. To reduce these effects, reduce the amount of OnEx Solution 4 to one-tenth the recommended volume (100 µl/liter medium).

Chemically defined culture media for autoinduction and Se-Met labeling

Overnight Express System 2 medium with Se-Met
Aseptically combine the following reagents in order[1]
Per liter:
1 ml OnEx Solution 3
1 ml OnEx Solution 4[2]
20 ml OnEx™ Solution 1
50 ml OnEx Solution 2
20 ml OnEx Solution 5
1 ml OnEx Solution 6
5 ml Se-Met (25 mg/ml)[3]
884 ml sterile deionized water

[1]When using a *metE* minus host strain [i.e., B834(DE3)], adding a final concentration of 100nM vitamin B12 to the medium significantly increases the yield of target protein (1).

[2]The trace metals present in OnEx Solution 4 may affect certain applications. To reduce these effects, reduce the amount of OnEx Solution 4 to one-tenth the recommended volume (100 µl/liter medium).

[3]Selenomethionine solution is not provided in the kit.

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Cell culture guidelines

These conditions may require optimization depending on the expression system, target protein, host strain, growth medium, temperature, culture volume, and orbital-shaking incubator used. The following protocols is are based on BL21(DE3) cell culture.

Note: *It is important to grow cells to stationary phase when using the Overnight Express Systems. See "Additional Guidelines" for more information.*

Prepare Overnight Express Autoinduction **System 1** medium aseptically by adding 0.02 vol OnEx™ Solution 1, 0.05 vol OnEx Solution 2, and 0.001 vol OnEx Solution 3 to 1 vol sterile glucose-free medium. Add appropriate antibiotics for the host strain and plasmid.

Prepare Overnight Express Autoinduction **System 2** medium aseptically by adding 0.02 vol OnEx™ Solution 1, 0.05 vol OnEx Solution 2, 0.001 vol OnEx Solution 3, 0.001 vol OnEx Solution 4, 0.02 vol OnEx Solution 5, and 0.02 vol OnEx Solution 6 to 1 vol sterile deionized water. Add appropriate antibiotics for the host strain and plasmid.

Prepare Overnight Express Autoinduction **System 2 (Se-Met labeling)** medium aseptically by adding 0.02 vol OnEx™ Solution 1, 0.05 vol OnEx Solution 2, 0.001 vol OnEx Solution 3, 0.001 vol OnEx Solution 4, 0.02 vol OnEx Solution 5, and 0.001 vol OnEx Solution 6 to 1 vol sterile deionized water. Add .005 vol Se-Met (25 mg/ml stock) and appropriate antibiotics for the host strain and plasmid.

Tube or flask cultures

Inoculate Overnight Express System medium plus appropriate antibiotics with 0.001 volume of a glycerol stock or with isolated colonies (1 colony/tube, 10–20 colonies/flask) from plates incubated overnight at 37°C. Incubate cultures for approximately 16 h with shaking at 300 rpm.

The following culture volumes and vessels are suggested to achieve appropriate aeration.

Culture volume	Vessel
0.5 ml	12 × 75 mm sterile snap-cap tube (VWR International, Cat. No. 60819-728)
2 ml	17 × 100 mm sterile snap-cap tube (VWR International, Cat. No. 60819-761)
10 ml	125-ml Erlenmeyer flask
30 ml	250-ml Erlenmeyer flask
100 ml	500-ml baffled flask
200 ml	1-L baffled flask
500 ml	2.8-L baffled flask

96-well or 24-well plate cultures

Inoculate Overnight Express System medium plus appropriate antibiotics with 0.001 volume of a glycerol stock or with an isolated colony (1 colony/well) from plates grown overnight at 37°C. Cover 96-well plates with an air-permeable sealer and incubate at 37°C, shaking at 300 rpm for approximately 16 h. Cover 24-well plates with BugStopper™ Venting Capmats (VWR International, Cat. No. 14217-208) and incubate at 37°C, shaking at 200 rpm for approximately 16 h.

The following culture volumes and vessels are recommended to achieve appropriate aeration.

Culture volume	Vessel
1 ml	Sterile 96-Well Deep Well Cultures Plates with Sealers (Cat. No. 71111-3)
5 ml	24-well culture plates (VWR International, Cat. No. 13503-190)

Additional Guidelines

Glycerol stock preparation: When growing cultures to prepare glycerol stocks, we recommend the addition of 0.5% glucose to a glucose-free medium (e.g., TB, LB broth, or 2X YT) to maintain plasmid stability. Grow the cells to an OD_{600} of 0.6–0.8 and add 0.1 vol of sterile 80% glycerol. Mix well and store at –70°C.

Aeration: Efficient growth to saturation and utilization of carbon sources provided by OnEx™ Solution 1 requires vigorous agitation and proper aeration. Optimized culture volume:vessel dimension ratio is required to achieve proper aeration.

Temperature and length of incubation: It is important to grow the cells to stationary phase when using the Overnight Express Systems. Using the cell culture guidelines above, stationary phase is usually reached as quickly as 8–10 hours, if the cultures are incubated at 37°C. When lower incubation temperatures are used, saturation may only be reached by incubation for 24 hours or more. Continued incubation for several hours after stationary phase appears to have no deleterious effects.

Growth and induction at 25°C or 30°C may be optimal if you want to export the target using the signal sequence leaders present in a number of pET vectors or improve the yield of soluble protein.

Bacterial strains: Because lactose is used for induction, expression hosts should produce functional *lac* permease (encoded by the *lacY* gene) and β-galactosidase (encoded by the *lacZ* gene) for consistent results in both complex and defined media. *lacY* mutant strains will not efficiently transport lactose for induction and *lacZ* mutants will not convert a portion of the transported lactose into the allolactose inducer. Elevated levels of target gene expression in *lacY* and *lacZ* mutant strains may occur as cells approach stationary phase in some complex media. However, this induction may vary depending upon medium composition, cell growth stage, and nutrient availability, all of which affect pH and the levels of cyclic AMP and acetate (4).

If using a plasmid with a T7*lac* promoter for expression, a host strain that does not contain a pLysS plasmid is recommended [i.e., BL21(DE3)]. The combination of the T7 lysozyme expressed by the pLysS plasmid and the *lac* repressor encoded by pET vectors carrying T7*lac* promoter results in significantly reduced levels of protein expression when using the Overnight Express Autoinduction Systems. When the "plain" T7 promoter is used, the low level of lysozyme provided by pLysS has little effect on expression of target proteins.

Expression vectors: Overnight Express Autoinduction Systems are compatible with pET bacterial expression vectors and other IPTG-inducible bacterial expression systems.

References

1. Studier, F. W. Personal communication.
2. Grabski, A., Mehler, M., and Drott, D. (2003) *in**Nova**tions* **17**, 3–6.
3. (2003) *inNovations* **18**, 26.
4. Grossman, T. H., Kawasaki, E. S., Punreddy, S. R., and Osburne, M. S. (1998) *Gene* **209**, 95–103.

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