

Overnight Express[™] Autoinduction Systems

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About the Kits

Overnight Express Instant TB Medium	1 EasyPak	71491-3
	5 EasyPaks	71491-4
	1 kg	71491-5
Overnight Express™ Autoinduction System 1	1 kit	71300-3
	1 kit	71300-4
Overnight Express Autoinduction System 2	1 kit	71366-3
	1 kit	71366-4

Description

The Overnight Express Autoinduction Systems are designed for high-level protein expression with pET and other IPTG-inducible bacterial expression systems without the need to monitor cell growth (1). Cell mass and target protein yield are often increased several-fold as compared with conventional protocols using induction with IPTG. The method is based on media components that are metabolized differentially to promote growth to high density and automatically induce protein expression from *lac* promoters. The Overnight Express Autoinduction Systems are extremely convenient for routine expression of proteins in multiple cultures in either complex (System 1 and Instant TB Medium) 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.

Overnight Express Instant TB Medium is a complete granulated culture medium supplied in two formats. EasyPak is an aluminum foil pouch containing 60 g granulated medium sufficient for 1 L culture. Just add the EasyPak contents to 1 L water, supplement with 10 ml glycerol, and microwave for a few minutes. In addition, the Overnight Express Instant TB Medium is supplied in 1 kg bottles. The granules ensure rapid and uniform dissolution in water, preventing clumping of the medium and inhalation of the airborne powder.

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 Luria-Bertani (LB) broth, Terrific 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).

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The Overnight Express™ Systems 1 and 2 kits provide sufficient reagents for 1 liter or 5 liters of media.

Components

Overnight Express System 1

- 20 or 5 × 20 ml OnEx™ Solution 1
- 50 or 250 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 250 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 Overnight Express Instant TB Medium at room temperature and protect from moisture. Store OnEx Solution 1 at room temperature until opened; store at 4°C once opened. Store OnEx Solutions 2–4 at room temperature. Store OnEx Solutions 5 and 6 at –20°C.

Notes: Overnight Express Instant TB Medium containers should be closed tightly after use. Absorption of water leads to changes in pH and eventually clumping. If clumps form, the medium has undergone chemical changes and the medium should be discarded.

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

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Overnight Express™ Instant TB Medium

Absorption of water by Overnight Express Instant TB Medium leads to changes in pH and eventually to clump formation. If the pH changes after prolonged storage, the pH can be adjusted (see below). However, medium that has formed clumps may have undergone chemical changes and should be discarded. Therefore, we recommend preparing all of the contents of an EasyPak as soon as the package is opened. DO NOT rehydrate portions of an EasyPak. Bottles of the dry medium should be tightly closed after use to prevent entry of moisture.

1. Pour the entire EasyPak (60 g) contents into a 2-L glass container or measure the appropriate amount of Overnight Express Instant TB Medium (60 g/L) and place in a vessel at least twice the final volume.
2. Add 1 L deionized water and 10 ml glycerol per 60 g Overnight Express Instant TB Medium.
3. Swirl gently until the medium is dissolved.
4. **Optional:** Divide the rehydrated Overnight Express Instant TB Medium into final culture volumes.

Note: Proper aeration is important for efficient growth and induction. For vessel size recommendations, see Cell Culture Guidelines, page 8

5. Heat the medium in a microwave oven on high power setting until bubbles start to appear (usually 2–3 min when using a 1500 W microwave oven per 500 ml medium). Continue to microwave for 15–30 s after bubbles start to appear. DO NOT let the medium boil over.

Notes: Microwave irradiation supplied by a standard carousel microwave oven offers an efficient, effective alternative for sterilization of prepared media (4).

Overnight Express Instant TB Medium, like all media, is heat-sensitive. Do not heat any longer than necessary.

If the vessel containing the medium is too large for a microwave, the medium can be autoclaved.

6. Set the vessel on a bench top and allow the medium to cool to room temperature.
7. Use immediately or store covered at 4°C until use.

Note: Rehydrated Overnight Express Instant TB Medium that has been prepared by heating in a microwave can be stored up to one week at 4°C before use. Warm to culture temperature before inoculation.

8. Add appropriate antibiotics for the host strain and plasmid prior to inoculation.

pH adjustment

Overnight Express Instant TB Medium should be pH 6.9 ± 0.2 . If the pH has changed after prolonged storage, adjust the pH using the following protocol.

1. Remove a sample (i.e., 50 ml) of the reconstituted culture medium.
2. Adjust the pH to 6.9 by adding 1 N or 0.1 N HCl or NaOH.
3. Calculate the volume of HCl or NaOH to adjust the pH of the remaining prepared culture medium.
4. Under sterile conditions, add the calculated volume of HCl or NaOH under sterile conditions.

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Overnight Express™ Autoinduction System 1

Culture media

Overnight Express System 1 is compatible with glucose-free media such as TB, LB broth, and 2X YT.

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.

Overnight Express System 1 medium
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

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Overnight Express™ Autoinduction System 2

Chemically defined culture medium for autoinduction

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.

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 deionized water

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

²The 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/L medium).

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Chemically defined culture medium for autoinduction and Se-Met labeling

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 0.005 vol Se-Met (25 mg/ml stock) and appropriate antibiotics for the host strain and plasmid.

Overnight Express System 2 medium with Se-Met

Aseptically combine the following reagents in order¹

Per liter:

1 ml OnEx Solution 3

1 ml OnEx Solution 4²

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

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

²The 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/L medium).

³L-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 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.

Tube or flask cultures

Inoculate Overnight Express System medium, plus appropriate antibiotic(s), with an isolated colony from plates grown overnight at 37°C, or with 0.001 volume of a glycerol stock. Inoculation of flask cultures greater than 30 ml or large volume fermentations should employ multistaging by generating a 5% (v/v) inoculum. Start the culture by single colony inoculation of 2 ml Overnight Express System medium, incubate with shaking 300 rpm to an OD₆₀₀ of approximately 0.5, and stage this 5% inoculum procedure to obtain the desired volume. Several stages and flasks of increasing size filled with medium (10–20% of the flask volume) may be required. The staging procedure will minimize the shock induced lag phase in growth caused by transfer of a small inoculum to a larger volume of fresh medium and diffusion of vitamins, minerals and cofactors from the cells (6). Incubate cultures for approximately 16 h with shaking at 300 rpm.

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

Culture volume	Vessel
0.5 ml	12 mm × 75 mm sterile snap-cap tube (VWR International, Cat. No. 60819-728)
2 ml	17 mm × 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)

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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 OD₆₀₀ 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.

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

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 (5).

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.

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