Overnight Express™ Instant LB and TB Media

Table of Contents

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Europe

France	**Germany**	**Ireland**	**United Kingdom**	**All other European Countries**
Freephone 0800 126 461	Freecall 0800 100 3496	Toll Free 1800 409 445	Freephone 0800 622 935	+44 115 943 0840

techservice@merckbio.eu

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

About the Kits

Overnight Express™ Instant LB Medium	1 EasyPak	71757-3
	5 EasyPaks	71757-4
	1 kg	71757-5
Overnight Express Instant TB Medium	1 EasyPak	71491-3
	5 EasyPaks	71491-4
	1 kg	71491-5

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 Instant LB and TB Media are extremely convenient for routine expression of proteins in multiple cultures in complex media and ideal for high-throughput parallel analysis of protein expression, solubility, and purification from multiple expression clones.

Overnight Express Instant LB or TB Media are complete granulated culture media supplied in two formats. The EasyPak is an aluminum foil pouch containing LB or TB 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 LB and TB Media are available in 1 kg bottles (use 45 g/L LB or 60 g/L TB). The granules ensure rapid and uniform dissolution in water, prevent clumping of the medium and significantly reduce inhalation of airborne powder.

Storage

Store Overnight Express Instant LB and TB Media at room temperature and protect from moisture.

Notes: *Overnight Express Instant LB and 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.*

Overnight Express™ Instant LB and TB Medium Preparation

Absorption of water by Overnight Express Instant LB or TB Media 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 contents into a 2-L glass container or measure the appropriate amount of Overnight Express Instant LB (45 g/L) or TB (60 g/L) Medium and place in a vessel at least twice the final volume.
2. Add 1 L deionized water and 10 ml glycerol per 45 g Overnight Express Instant LB or 60 g Overnight Express Instant TB Medium.
3. Swirl gently until the medium is dissolved.
4. **Optional:** Divide the rehydrated Overnight Express Instant LB or TB Medium into final culture volumes.

*Note: Proper aeration is important for efficient growth and induction. For vessel size recommendations, see "*Cell Culture Guidelines*" (p 4).*

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.
6. Alternatively, the medium can be sterilized by autoclaving for the shortest liquid autoclave cycle, i.e., approximately 15-20 minutes. After the cycle is complete, remove immediately to cool. Please note, if the medium is heated for too long or not removed promptly, carmelization may occur (i.e., the medium turns a brown color).

Notes: Microwave irradiation supplied by a standard carousel microwave oven offers an efficient, effective alternative for sterilization of prepared media (2).

Overnight Express Instant LB and TB Media, like all media, are heat-sensitive. Do not heat any longer than necessary.

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

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

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

*Note: Please see "*Additional Guidelines*" (p 5) for more information on antibiotic concentrations.*

pH adjustment

Overnight Express Instant LB or TB Medium should be pH 6.9 $\pm$ 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. Add the calculated volume of HCl or NaOH under sterile conditions.

Cell Culture Guidelines

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

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

*It is important to grow cells to stationary phase when using Overnight Express Media. See "*Additional Guidelines*" (p 5) for more information.*

Tube or flask cultures

Prepare Overnight Express Instant LB or TB, plus appropriate antibiotic(s), and place in appropriate sized vessel. For final culture volumes less than 30 ml, inoculate with an isolated colony from plates grown overnight at 37ºC, or with 0.001 volume of a glycerol stock. For final culture volumes greater than 30 ml or for large volume fermentations, inoculate with 1-5% (v/v) of a non-induced, log phase starter culture.

Incubate cultures until growth is saturated with shaking at 300 rpm. Saturation will generally be reached after 16 h at 37ºC; see "Additional Guidelines" (p 5) for more information. Harvest cells from saturated, stationary phase culture for analysis and protein preparation.

Preparation of Starter Cultures

For autoinducing cultures of final volumes greater than 30 ml, a non-induced, log phase starter culture should be prepared to serve as an inoculum (4). Use a single colony to inoculate plain LB or TB + 0.5% glucose (or any other non-inducing medium). Incubate with shaking at 300 rpm until OD_{600} is between 0.5 and 1.0. This starter culture can be used immediately, or stored at 4ºC for up to 24 hr.

Note: *If non-inducing medium (plain LB or TB) is not available, Overnight Express Instant LB or TB may be used, but the culture must not exceed OD_{600}=0.5 as it is essential to avoid induction.*

Inoculum for very large scale expression cultures can be prepared by staging (e.g., 30 ml starter culture used to inoculate 2 L starter culture to be inoculum for 100 L expression culture). Several stages and flasks of increasing size filled with medium (10–20% of the flask volume) may be required during inoculum preparation. 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 Instant LB or TB 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, or with 0.001 volume of a glycerol stock. 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

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

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. We do not recommend the use of autoinducing media for preparation of glycerol stocks.

Aeration: Efficient growth to saturation and utilization of carbon sources provided by Overnight Express Media 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 Media. 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 (3). Overnight Express Media are compatible with host strains such as BL21, Rosetta™, Rosetta 2, and their DE3 lysogen derivatives. The following host strains and their derivatives are not compatible with Overnight Express: NovaBlue, Tuner™, Origami™ 2, Origami B, RosettaBlue™, Rosetta-gami™ 2 and Rosetta-gami B.

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 Media When the "plain" T7 promoter is used, the low level of lysozyme provided by pLysS has little effect on expression of target proteins.

Antibiotics: With ampicillin-resistant pET vectors, a final working concentration of 50 µg/ml ampicillin or carbenicillin is suggested for selection. However, with kanamycin resistant vectors, selection with kanamycin is much less effective in autoinduction media (1). Therefore, we recommend using 100 µg/ml kanamycin with pET vectors encoding kanamycin resistance with our Overnight Express Autoinduction media.

Note: *Please visit www.novagen.com/onexpress for more information on Overnight Express Media.*

References

1. Studier, F. W. (2005) *Protein Expression and Purification.* **41**, 207-234.
2. Border, B. G., Rice-Spearman L. (1999) *Clin. Lab. Sci.* **12**, 156–160.
3. Grossman, T. H., Kawasaki, E. S., Punreddy, S. R., and Osburne, M. S. (1998) *Gene* **209**, 95–103.
4. Hunt, G.R., and Stieber, R.W., (1986) *Manual of Industrial Microbiology and Biotechnology.* F (Demain, A.L., and Solomon, N.A., Eds) pp. 32–40, ASM. Washington, D.C.

Limited License

The AutoInduction Media Technology embodied in the Overnight Express™ Instant Media is based on technology developed at Brookhaven National Laboratory under contract with the U. S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a non-exclusive license for use of this technology, including the enclosed materials, based upon the following assurance:

These materials are to be used for research purposes only. A separate license is required for any commercial manufacture or use, including the manufacture of protein products for use in the screening of compound libraries. Information about commercial licenses may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Bldg. 475D, P. O. Box 5000, Upton, New York 11973-5000, Telephone: (631) 344-7134.

You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license.