Overnight Express™ Autoinduction NMR Media

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Description

Overnight Express™ Autoinduction media are designed to promote high-level protein expression with pET and other IPTG-inducible bacterial expression systems without adding inducer or requiring cell density monitoring to determine appropriate induction time (1–4). Overnight Express™ Auto induction NMR Media comprise of three kits for assembling autoinducing liquid media used to produce isotopically-labeled proteins for NMR spectroscopy (see Table 1 below). NMR Medium–Optimization may be used first to determine ideal culture conditions for high-level protein expression in absence of isotopic label. NMR Medium–^{15}N enables efficient incorporation of ^{15}N for single-label NMR analysis and to assess suitability for structural studies. NMR Medium–^{15}N, ^{13}C is used to incorporate both ^{15}N and ^{13}C into target proteins for structural determination.

For autoinduction of expression, Overnight Express™ NMR Media contain the following four concentrated, sterile solutions:

- **OnEx™ NMR Solution 1** (no label or single [*U*-^{15}N] label) or **OnEx™ Dual-NMR Solution 1** (dual [*U*-^{15}N, *U*-^{13}C] label) each contain a blend of carbon sources for tightly regulated, uninduced growth to high cell density followed by induction of target protein expression.
- **OnEx™ NMR Solution 2** is a concentrated buffer for maintaining neutral pH throughout metabolic acid production.
- **OnEx™ Solution 3** contains magnesium necessary for cultures to attain high cell density.
- **OnEx™ Solution 4** includes trace metals to avoid growth limitations from mineral deficiencies and to satisfy the requirements of metalloproteins expressed to high levels.

The addition of these four components and the appropriate isotopic label(s) to sterile water creates a chemically defined medium capable of promoting growth to high cell density, autoinduction of expression, and efficient labeling of target proteins (5).

Overnight Express™ Auto induction NMR Media also include reagents to make **non-inducing starter medium** for staged growth without expression prior to labeling. Additionally, this chemically defined medium may be used for stable storage of expression plasmid-containing bacterial strains. Non-inducing starter medium is assembled with the same magnesium (OnEx™ Solution 3) and trace metals (OnEx™ Solution 4) solutions used in the autoinduction media, as well as three additional solutions: **OnEx™ Solution 2**, a concentrated buffer that maintains pH and supplies necessary nitrogen, **OnEx™ NMR Solution 5**, an amino acid mixture, and **OnEx™ NMR Solution 6**, a non-inducing carbon source.

The Overnight Express™ Auto induction NMR Media–Optimization and –^{15}N, ^{13}C kits provide sufficient reagents to prepare 1 liter of autoinduction medium and 0.2 liters of non-inducing starter medium. The NMR Medium–^{15}N kits include reagents to prepare 1 or 5 liters of autoinduction medium and 0.2 or 1 liter of non-inducing starter medium.

Table 1. Overnight Express™ Autoinduction NMR Media Kits Selection Guide

Catalog Number	Overnight Express™ Auto induction Kit	Intended Purpose
71760-3	NMR Medium–Optimization	Optimize culture conditions
71759-3 71759-4	NMR Medium–^{15}N	Single label to assess suitability for further analysis
71789-3	NMR Medium–^{15}N, ^{13}C	Dual label to determine structure

Components

Overnight Express™ Autoinduction NMR Medium–Optimization

- 20 ml OnEx™ NMR Solution 1
- 10 ml OnEx™ Solution 2
- 50 ml OnEx™ NMR Solution 2

- 3 × 1 ml OnEx™ Solution 3
- 1 ml OnEx™ Solution 4
- 4 ml OnEx™ NMR Solution 5
- 5 ml OnEx™ NMR Solution 6
- 2.7 g Ammonium Chloride

Overnight Express™ Auto induction NMR Medium–^{15}N

- 20 or 100 ml OnEx™ NMR Solution 1
- 10 or 50 ml OnEx™ Solution 2
- 50 or 250 ml OnEx™ NMR Solution 2
- 3 × 1 ml or 11 ml OnEx™ Solution 3
- 1 or 2 × 1 ml OnEx™ Solution 4
- 4 or 20 ml OnEx™ NMR Solution 5
- 5 or 25 ml OnEx™ NMR Solution 6
- 2.7 g or 5 × 2.7 g [^{15}N]Ammonium Chloride

Overnight Express™ Auto induction NMR Medium–^{15}N, ^{13}C

- 20 ml OnEx™ Dual-NMR Solution 1
- 10 ml OnEx™ Solution 2
- 50 ml OnEx™ NMR Solution 2
- 3 × 1 ml OnEx™ Solution 3
- 1 ml OnEx™ Solution 4
- 4 ml OnEx™ NMR Solution 5
- 5 ml OnEx™ NMR Solution 6
- 2.7 g [^{15}N]Ammonium Chloride
- 0.5 g [*U*-$^{13}C_6$]D-Glucose

Storage

Store OnEx™ NMR Solution 5 at –20˚C. Store OnEx™ NMR Solution 1, OnEx™ Dual-NMR Solution 1, and OnEx™ NMR solution 6 at 4°C. Store OnEx™ Solution 2, OnEx™ NMR Solution 2, OnEx™ Solution 3, OnEx™ Solution 4, Ammonium Chloride, [^{15}N]Ammonium Chloride, and [*U*-$^{13}C_6$]D-glucose at room temperature.

Note: All OnEx™ Solutions are supplied as sterile solutions and require use of aseptic techniques.

Reagents Required but not Supplied

- [$^{13}C_3$]Glycerol (Cat. No. CLM1510EMD)
- Appropriate antibiotics
- LB agar plates containing 0.5% (w/v) glucose
- LB broth containing 0.5% (w/v) glucose
- Sterile deionized water

Media Preparation

Overnight Express™ Autoinduction NMR Medium–Optimization

Prepare Overnight Express™ Autoinduction NMR Medium–Optimization by aseptically pipeting OnEx™ NMR Solution 1 (provided as a 50X concentrated stock), OnEx™ NMR Solution 2 (20X stock), OnEx™ Solution 3 (500X stock), OnEx™ Solution 4 (5000X stock) and NH_4Cl (provided as a solid, prepare 1M stock and add to 50 mM final concentration) in sterile deionized water. NH_4Cl is provided in solid form; prepare a sterile 1 M stock solution prior to assembling media. Once medium is assembled (see table below), add appropriate antibiotics for the host strain and plasmid.

Note: The order of component addition is important to avoid precipitation of the magnesium and metal salts.

Overnight Express™ Auto induction NMR Medium–Optimization Per liter[a]:		
Order of addition	**Component**	**Volume**
1	Sterile deionized water	800 ml
2	OnEx™ Solution 3	2 ml
3	OnEx™ Solution 4	0.2 ml
4	OnEx™ NMR Solution 1	20 ml
5	OnEx™ NMR Solution 2	50 ml
6	1 M NH_4Cl[b]	50 ml
7	Sterile deionized water	To 1.0 L[c]

[a]Other volumes may be prepared as long as the relative ratios of the solutions are maintained.

[b]Make a 1M stock solution by dissolving 2.7 g NH_4Cl in deionized water and filter-sterilize. The sterile stock solution can be stored at 4°C.

[c]When using *metE* mutant host strains (e.g., B834), supplement medium with 100–200 nM vitamin B_{12} (Cat. No. 6791). Prepare a 5 mM stock in 100% ethanol, dilute to 200 µM with water, and filter-sterilize. The 200 µM stocks are stable for one week when stored at 4°C in the dark.

Note: Overnight Express™ Auto induction NMR Medium–Optimization should be prepared on the day of use.

Caution: Do NOT autoclave the individual solutions or the assembled medium. If contaminated during preparation, the assembled medium may be filter-sterilized.

Overnight Express™ Autoinduction NMR Medium–^{15}N

Prepare Overnight Express™ Autoinduction NMR Medium–^{15}N by aseptically pipeting OnEx™ NMR Solution 1 (provided as a 50X concentrated stock), OnEx™ NMR Solution 2 (20X stock), OnEx™ Solution 3 (500X stock), OnEx™ Solution 4 (5000X stock) and [^{15}N]NH_4Cl (provided as a solid, prepare 1M stock and add to 50 mM final concentration) in sterile deionized water. [^{15}N]NH_4Cl is provided in solid form; prepare a sterile 1 M stock solution prior to assembling media. Once medium is assembled (see table below), add appropriate antibiotics for the host strain and plasmid.

Note: The order of component addition is important to avoid precipitation of the magnesium and metal salts.

Overnight Express™ Auto induction NMR Medium–^{15}N Per liter[a]:

Order of addition	Component	Volume
1	Sterile deionized water	800 ml
2	OnEx™ Solution 3	2 ml
3	OnEx™ Solution 4	0.2 ml
4	OnEx™ NMR Solution 1	20 ml
5	OnEx™ NMR Solution 2	50 ml
6	1 M [^{15}N]NH_4Cl[b]	50 ml
7	Sterile deionized water	To 1.0 L[c]

[a]Other volumes may be prepared as long as the relative ratios of the solutions are maintained.

[b]Make a 1M stock solution by dissolving 2.7 g [^{15}N]NH_4Cl in deionized water and filter-sterilize. The sterile stock solution can be stored at 4°C.

[c]When using *metE* mutant host strains (e.g., B834), supplement medium with 100–200 nM vitamin B_{12} (Cat. No. 6791-5G). Prepare a 5 mM stock in 100% ethanol, dilute to 200 µM with water, and filter-sterilize. The 200 µM stocks are stable for one week when stored at 4°C in the dark.

Note: Overnight Express™ Auto induction NMR Medium–^{15}N should be prepared on the day of use.

Caution: Do NOT autoclave the individual solutions or the assembled medium. If contaminated during preparation, the assembled medium may be filter-sterilized.

Overnight Express™ Autoinduction NMR Medium–^{15}N, ^{13}C

Prepare Overnight Express™ Autoinduction NMR Medium–^{15}N, ^{13}C by aseptically pipeting OnEx™ Dual-NMR Solution 1 (provided as a 50X concentrated stock), OnEx™ NMR Solution 2 (20X stock), OnEx™ Solution 3 (500X stock), OnEx™ Solution 4 (5000X stock), [^{15}N]NH_4Cl (provided as a solid, prepare 1M stock and add to 50 mM final concentration), [$^{13}C_3$]Glycerol (0.5% (w/v) final concentration), and [*U*-^{13}C]D-glucose (provided as a solid, prepare 2.5% solution and add to 0.05% (w/v) final concentration) in sterile deionized water. [^{15}N]NH_4Cl is provided in solid form; prepare a sterile 1 M stock solution prior to assembling media. Similarly, [*U*-$^{13}C_6$]D-glucose is available in solid form (purchased separately); prepare a sterile 2.5% stock solution. Once medium is assembled (see table below), add appropriate antibiotics for the host strain and plasmid.

Note: The order of component addition is important to avoid precipitation of the magnesium and metal salts.

Overnight Express™ Auto induction NMR Medium–^{15}N, ^{13}C
Per liter[a]:

Order of addition	Component	Volume
1	Sterile deionized water	800 ml
2	OnEx™ Solution 3	2 ml
3	OnEx™ Solution 4	0.2 ml
4	OnEx™ Dual NMR Solution 1	20 ml
5	OnEx™ NMR Solution 2	50 ml
6	1 M [^{15}N]NH_4Cl[b]	50 ml
7	[$^{13}C_3$]Glycerol[c]	5 g
8	2.5% (w/v) [*U*-$^{13}C_6$]D-glucose[d]	20 ml
9	Sterile deionized water	To 1.0 L[e]

[a]Other volumes may be prepared as long as the relative ratios of the solutions are maintained.

[b]Make a 1M stock solution by dissolving 2.7 g [^{15}N]NH_4Cl in deionized water and filter-sterilize. The sterile stock solution can be stored at 4°C.

[c]**Not included in the kit.** Sold in liquid form (Cat. No. CLM1510EMD). Use entire 5 g per liter of medium.

[d]Prepare a 2.5% (w/v) solution by combining 0.5 g [*U*-$^{13}C_6$]D-glucose with 20 ml sterile deionized water.

[e]When using *metE* mutant host strains (e.g., B834), supplement medium with 100–200 nM vitamin B_{12} (Calbiochem Cat. No. 6791). Prepare a 5 mM stock in 100% ethanol, dilute to 200 µM with water, and filter-sterilize. The 200 µM stocks are stable for one week when stored at 4°C in the dark.

Note:Overnight Express™ Auto induction NMR Medium–^{15}N, ^{13}C should be prepared on the day of use.

Caution: Do NOT autoclave the individual solutions or the assembled medium. If contaminated during preparation, the assembled medium may be filter-sterilized.

Non-inducing starter medium

Prepare non-inducing starter medium by aseptically pipeting OnEx™ Solution 2 (provided as a 20X concentrated stock), OnEx™ Solution 3 (500X stock, used at 0.5X – see table below), OnEx™ Solution 4 (5000X stock), OnEx™ NMR Solution 5 (50X stock), and OnEx™ NMR Solution 6 (40X stock) in sterile deionized water. Once combined (see table below), add appropriate antibiotics for the host strain and plasmid.

Note: The order of component addition is important to avoid precipitation of the magnesium and metal salts.

Non-inducing starter medium Per 200 ml[a]:		
Order of addition	**Component**	**Volume**
1	Sterile deionized water	150 ml
2	OnEx™ Solution 3[b]	0.2 ml
3	OnEx™ Solution 4	0.04 ml
4	OnEx™ NMR Solution 5	4 ml
5	OnEx™ NMR Solution 6	5 ml
6	OnEx™ Solution 2[c]	10 ml
7	Sterile deionized water	To 200 ml

[a]Other volumes may be prepared as long as the relative ratios of the solutions are maintained.

[b]Final concentration is 0.5X.

[c]**Use OnEx™ Solution 2, NOT OnEx™ NMR Solution 2**.

Note: Non-inducing starter medium should be prepared on the day of use.

Caution: Do NOT autoclave the individual solutions or the assembled medium. If contaminated during preparation, the assembled medium may be filter-sterilized.

Cell Culture Guidelines

Optimal culture conditions depend upon the expression plasmid, target protein, and host cell strain. The following protocol is based on a 500-ml B834(DE3) cell culture. Obtaining high-level expression of soluble target protein may require adjusting several parameters including temperature, length of incubation, culture and vessel volume, and type and speed of orbital-shaking incubator (4). We recommend first performing small-scale expression experiments using the Overnight Express™ Autoinduction NMR Medium–Optimization (i.e., without isotopic label) to determine the best conditions for expression.

Preparation of Starter Culture

1. Transform expression plasmid into the desired host strain and plate on LB agar plus 0.5% (w/v) glucose plates supplemented with appropriate antibiotic(s). Incubate overnight at 37°C.
   Alternatively: Streak out glycerol stock containing expression plasmid on LB agar plus 0.5% (w/v) glucose plates supplemented with antibiotic(s).
2. Inoculate 3.5 ml LB plus 0.5% (w/v) glucose and appropriate antibiotic(s) with a single, well-isolated colony.

   Tip: Prepare the starter culture using a fresh colony. Colonies from plates stored for more than 24 h often fail to support high-level expression of target protein. We recommend preparing multiple 3.5 ml starter cultures at this stage in case one fails to grow.
3. Incubate at 37°C with shaking at 275–300 rpm to an OD_{600} of 1.0–1.5 (approximately 3 h).

   Caution: Do NOT allow cultures grown in LB to reach saturation. LB and other complex media containing enzymatic digests of casein can promote unintended induction of target protein expression as glucose becomes depleted (4, 6). Additionally, as cultures approach saturation, the media may become sufficiently acidic to arrest growth and decrease viability.
4. Using aseptic technique, add 3 ml of the culture to 50 ml pre-warmed (37°C) non-inducing starter medium with appropriate antibiotic(s).
5. Incubate at 37°C with shaking at 275–300 rpm to OD_{600} 2.0–3.0 (approximately 4 h). Use immediately for inoculating autoinduction medium.

   Note: Alternatively, the starter culture may be prepared using non-inducing starter medium exclusively (i.e., substitute non-inducing starter medium for the LB at Step 2 above) (7). However, since growth is much slower, incubation times required to reach the appropriate culture densities will be significantly longer. For example, 3.5 ml non-inducing starter medium inoculated with a single colony may need 7–8 hours to reach OD_{600} 1.0–1.5. The subsequent 50 ml starter culture may then be incubated overnight.

Autoinduction in Overnight Express™ NMR Media

1. Equilibrate 500 ml Overnight Express™ Auto induction NMR (–Optimization, $-^{15}N$, or $-^{15}N$, ^{13}C) medium plus appropriate antibiotic(s) to 37°C.
2. Aseptically add 40 ml non-inducing starter culture to pre-warmed autoinduction medium.
3. Incubate at 37°C with shaking at 275–300 rpm for 16–24 h.

   Note: It is important to grow cells to stationary phase when using Overnight Express™ Auto induction NMR Media. See "Additional Guidelines" for more information.

   Note: For autoinduction at lower temperatures, we recommend an initial incubation at 37°C for 2 h, fol lowed by 16–24h incubation at 25°C (autoinduction below 25°C may re quire >24 h incubation for cells to reach stationary phase).
4. Harvest cells by centrifugation at >5,000 x *g* for 15 min at 4°C. Pellets may be stored at –70°C until ready for further processing.

Additional Guidelines

Glycerol stock preparation: To prepare glycerol stocks of bacterial strains containing expression plasmid, grow cells to log phase in non-inducing starter medium plus antibiotics. Add 0.1 vol sterile 80% glycerol. Mix well and store at –70°C.

Aeration: Efficient growth to saturation and utilization of carbon sources provided by OnEx™ NMR Solution 1 and OnEx™ Dual-NMR Solution 1 requires vigorous agitation and proper aeration. Optimized culture volume to vessel dimension ratio is required to achieve proper aeration. Media should not comprise more than 20% of the flask volume. We strongly recommend the use of baffled flasks for superior aeration.

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

Temperature and length of incubation: It is important to grow the cells to stationary phase with the Overnight Express™ Autoinduction Systems. Cultures incubated at temperatures below 37°C may require 24 h or more to reach saturation. Continued incubation for several hours after cultures reach stationary phase appears to have no deleterious effects.

For any given combination of expression plasmid, target protein and host cell strain, the optimal temperature and length of time for autoinduction must be determined empirically. Induction at 25°C or 30°C may improve the yield of soluble protein or the efficiency of transport to the periplasm when using signal sequence leaders present in a number of pET vectors.

Bacterial strains: Because lactose is used for autoinduction, **expression hosts must produce functional lac permease (encoded by the *lacY* gene) and β-galactosidase (encoded by the *lacZ* gene)**. *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.

Overnight Express™ Auto induction Systems may be used with B834, BL21, HMS174, Rosetta™, Rosetta™ 2, and their DE3 lysogen derivatives. *The following Novagen® host strains and their derivatives are **NOT** compatible with Overnight Express™:* NovaBlue, Tuner™, Origami™, Origami 2, Origami B, RosettaBlue™, Rosetta-gami™, Rosetta-gami™ 2, Rosetta-gami™ B, and BLR(DE3).

If using a plasmid with a T7*lac* promoter for expression, we recommend using a host strain that does not contain the pLysS plasmid. The combination of the T7 lysozyme expressed from the pLysS plasmid and the Lac repressor expressed from pET vectors carrying the T7*lac* promoter results in significantly reduced protein expression levels with the Overnight Express™ Auto induction Systems. When using the "plain" T7 promoter, the low level of lysozyme provided by pLysS has little effect on target protein expression.

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

References

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Limited License

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