EnPresso B Defined Nitrogen-free Starter Pack

EnPresso B Defined Nitrogen-free (B21200)

Components	**Tablets, blue bag:** Each bag contains two tablets composed of minerals, vitamins, trace elements and polysaccharide substrate	**4 bags**
	Reagent A (3000 U/L) Glucose-releasing agent	**1 mL**
Format	Each bag contains sufficient components for a 50 mL culture. Tablets and reagent are manufactured using standard aseptic techniques and filtration or gamma irradiation to ensure sterility.	
Storage	Store kit contents at 4-25°C. After opening, store Reagent A at 4-8°C.	
Shelf life	Expiration date is indicated on the box.	

Additional items needed

LB media (1 mL), cultivation tube or shake flask for pre-culture

Sterile solution of $^{15}NH_4Cl$ (40 g/L)

Sterile shake flask, 1000 mL

Antibiotics

Inducer such as IPTG

Anti-foaming agent, such as AntiFoam 204, when using Ultra Yield Flasks or other baffled (fluted) flasks

Note: For shake flask cultures, we recommend the use of Ultra Yield Shake Flasks with AirOtop Enhanced Seals. The improved aeration has been shown to further enhance the performance of EnPresso growth systems for bacterial cultures.

Description

EnPresso® B Defined Nitrogen-free is a pre-sterilized, chemically-defined growth system designed to increase the yield of ^{15}N-labelled proteins.
EnPresso growth systems provide optimal conditions for growth, metabolism and protein expression in microbial cultures. Protein yields are increased by enabling cultures to reach far higher cell densities than those achieved using conventional media.
By controlling growth rate and metabolism, a greater proportion of expressed protein can be correctly folded to improve solubility, minimize the risk of inclusion body formation, and ensure functionality of the final product.
EnPresso growth systems maintain pH, provide adequate minerals, vitamins and trace elements to support growth, and use proprietary EnBase® technology to ensure a constant, slow release of glucose from a polysaccharide substrate.

Recommended conditions

Culture volume: 10% of flask volume (20% only if using Ultra Yield flasks)

Shaking: 250 rpm, 25-50 mm amplitude. Use 25 mm amplitude for Ultra Yield Flasks.

Temperature pre-culture: 37°C

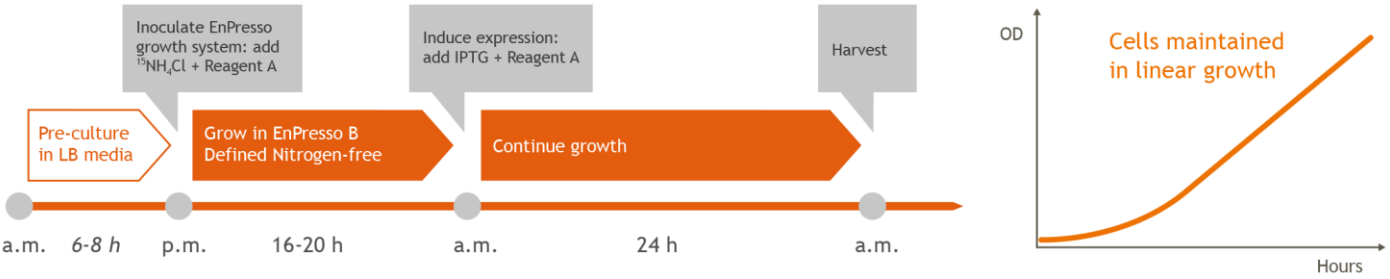
Temperature culture: 30°C

IMPORTANT NOTICE:
It is essential to follow the EnPresso B Defined Nitrogen-free protocol in detail. Using a fresh pre-culture, the correct shake speed, air-permeable closures and recommended cultivation times are critical to ensure success.
Never use aluminum foil or plastic lids during incubation.

Detailed protocol – see overleaf→

BioSilta Ltd.
8 Bramley Road
St. Ives
Cambridgeshire
PE27 3WS
United Kingdom

www.biosilta.com
Email:info@biosilta.com
Tel: +44 1480 400 535



EnPresso B Defined Nitrogen-free - from pre-culture to harvest within 2 days

Protocol for ^{15}N labeling in shake flasks (100 mL culture)

Day 1

1. Prepare an inoculum from a glycerol stock or use a single colony grown overnight on an agar plate. Inoculate 1 mL of LB medium containing antibiotics in a cultivation tube or shake flask.
2. Incubate with vigorous shaking at 37°C for 6 h or until the inoculum has reached OD 2 - OD 4.
3. Add aseptically into a sterile 1000 mL shake flask
    - 90 mL sterile water
    - Four tablets (two bags) of EnPresso B Defined Nitrogen-free
    - 6 mL $^{15}NH_4Cl$ (40 g/L, final concentration 2.5 g/L)

Note: 2.5 g/L final concentration of $^{15}NH_4Cl$ is recommended as a starting concentration. Concentrations as low as 1.0 g/L and as high as 4.0 g/L have also been used successfully.

4. Immediately shake vigorously at 37°C until the tablets have fully dissolved. After dissolution, small crystals of magnesium salts may occasionally be visible, but these will not affect performance.
5. Add required antibiotics. If using Ultra Yield Flasks, add anti-foaming agent, such as 10 µL AntiFoam 204 in 100 mL culture volume.
6. Inoculate with 1:100 of the pre-culture inoculum (1 mL).
7. Add 50 µL Reagent A (final concentration 1.5 U/L)
8. Close the flask securely.
9. Incubate overnight (16-20 h) at 30°C, 250 rpm.

Day 2

10. Add induction agent and 170 µL Reagent A (final concentration 5 U/L)
11. Continue incubation at 30°C, 250 rpm
12. Sample after 6 to 8 hours induction to evaluate protein production.
13. Continue incubation overnight.

Day 3

14. Harvest.

Note: This protocol has been optimized to enhance the growth of *E. coli*. For other tips and hints on how to achieve optimal performance when growing bacteria, visit www.biosilta.com

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BioSilta Ltd.
8 Bramley Road
St. Ives
Cambridgeshire
PE27 3WS
United Kingdom

www.biosilta.com
Email:info@biosilta.com
Tel: +44 1480 400 535