

EnPresso B Animal-free



EnPresso B Animal-free (B11101)

Components	Tablets, white bag:	20 bags
	Each bag contains two tablets composed of minerals, vitamins and trace elements, inorganic and organic nitrogen for pH control and polysaccharide substrate	
	Booster tablet, black bag	20 bags
	Each bag contains one tablet composed of extra nutrients and polysaccharide substrate	
	Reagent A (3000 U/L)	5 mL
	Glucose-releasing agent	
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 (2mL), cultivation tube or shake flask for pre-culture

Sterile shake flask, 500 mL or multiwell plates

Sterile water, 50 mL

Antibiotics

Inducer such as IPTG

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

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

Description

EnPresso[®] B is a pre-sterilized growth system designed to increase the yield of functional protein from *E. coli*-based expression systems. 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

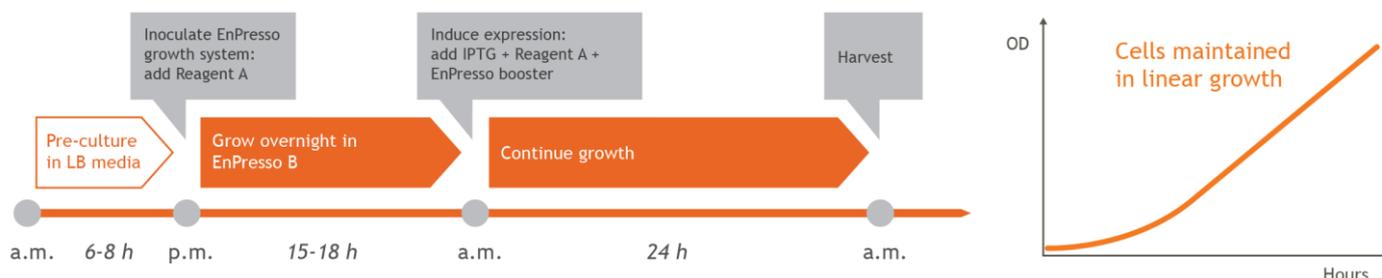
Temperature culture: 28-30°C

IMPORTANT NOTICE:

It is essential to follow EnPresso B Animal-free protocols 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 protocols overleaf →



EnPresso B growth system - from pre-culture to harvest within two days

Protocol for shake flasks - 50 mL culture

Day 1 - step 1

1. Prepare inoculum from a glycerol stock or use a single colony grown overnight on an agar plate. Inoculate 2 mL of LB medium containing antibiotics in a cultivation tube or shake flask.
2. Incubate at 37°C for 6-8 hours with vigorous shaking.

Day 1 - step 2

3. Add contents of one white bag (2 tablets) to 50 mL of sterile water in a sterile 500 mL shake flask.
4. Immediately shake vigorously to ensure tablets begin to dissolve.
5. Add required antibiotics. If using Ultra Yield Flasks, add anti-foaming agent such as 5 µL AntiFoam 204 per 50 mL culture volume.
6. Inoculate with 1:25 of the pre-culture inoculum (2 mL).
7. Add 25 µL Reagent A (final concentration 1.5 U/L)
8. Close the flask securely.
9. Incubate overnight (15-18 h) at 30°C, 250 rpm.

Day 2

10. Add induction agent, booster tablet (black bag) and 25 µL Reagent A. If using Ultra Yield Flasks, add 75 µL Reagent A.
11. Continue to incubate at 30°C, 250 rpm for a further 24 hours.

Day 3

12. Harvest.

Note: After complete dissolution of EnPresso B tablets, small crystals of magnesium salts may be visible, but these will not affect performance.

Note: After adding induction agent it may be possible to harvest after 6 hours. This can be beneficial when expressing a toxic protein.

Note: For tips and hints on how to maximize performance for a specific culture or for handling larger volumes, visit www.biosilta.com

Protocol for multiwell plates - 50 mL batch

Day 1 -step 1

1. Prepare inoculum from a glycerol stock or use a single colony grown overnight on an agar plate. Inoculate 2 mL of LB medium containing antibiotics.
2. Incubate at 37°C for 6-8 hours with vigorous shaking.

Day 1 - step 2

3. Add contents of one white bag (2 tablets) to 50 mL of sterile water in a sterile shake flask.
4. Immediately shake vigorously at 37°C until the tablets have fully dissolved. After dissolution small crystals of magnesium salts may be visible, but these will not affect performance.
5. Add required antibiotics and 25 µL Reagent A.
6. Mix and aliquot into microplate wells.
7. Inoculate each well with the pre-culture inoculum: 1:25.
8. Seal the plate with a porous membrane seal.
9. Incubate overnight (15-18 h) at 30°C; minimum shake speed 250 rpm with 25 mm offset or 200 rpm with 50 mm offset.

Day 2

10. Prepare a solution of booster tablet (black bag), Reagent A and induction agent:
 - a. Dissolve completely one booster tablet in 5 mL sterile water.
 - b. Add 25 µL Reagent A.
 - c. Add induction agent to give 10X required final concentration.
11. Aliquot solution into each well (1:10 of culture volume per well)
12. Continue incubation for 24 hours.

Day 3

13. Harvest.

Note: For tips and hints on how to maximize performance when growing bacteria with EnPresso B, visit www.biosilta.com